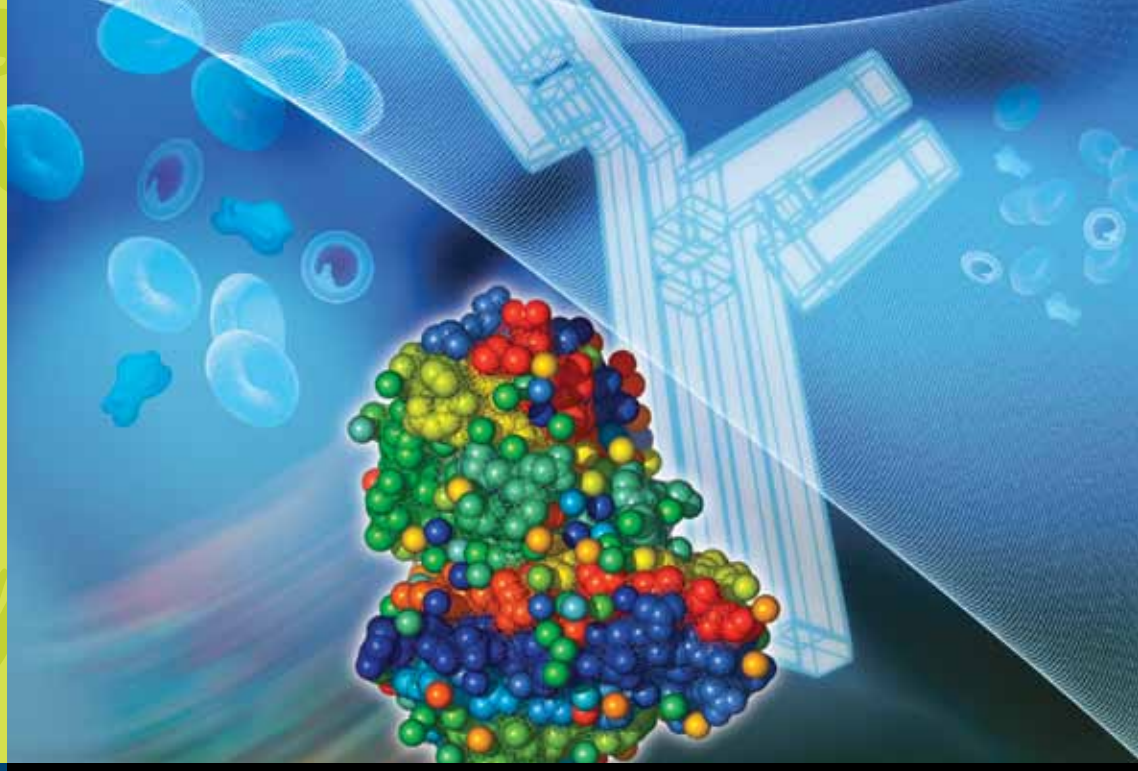

Thermo Scientific Pierce Antibody Production and Purification Technical Handbook

Version 2

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

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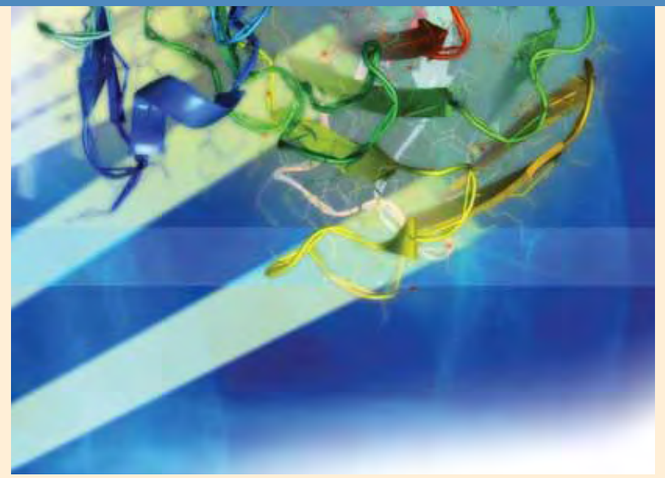
Antibody Overview



Introduction to Antibody Production, Purification and Modification

The ability of animal immune systems to produce antibodies capable of binding specifically to antigens can be harnessed to manufacture probes for detection of molecules of interest in a variety of research and diagnostic applications. No other current technology allows researchers to design and manufacture such highly specific molecular recognition tools.

Nearly all medical or cell biology researchers doing any kind of molecular analysis make use of antibody technology in one form or another. Depending upon the uniqueness of their research targets, these scientists will differ in the extent to which they concern themselves with antibody production and purification.

Antibodies are host proteins that are produced by the immune system in response to foreign molecules that enter the body. These foreign molecules are called antigens, and their molecular recognition by the immune system results in selective production of antibodies that are able to bind the specific antigen. Antibodies are made by B-lymphocytes and circulate throughout the blood and lymph where they bind to their specific antigen, enabling it to be cleared from circulation.

Several important features besides their high specificity make antibodies particularly conducive to development as probes. For example, except in those portions that determine antigen binding, antibodies share a relatively uniform and well-characterized protein structure that enables them to be purified, labeled and detected predictably and reproducibly by generalized methods.

Procedures for generating, purifying and modifying antibodies for use as antigen-specific probes were developed during the 1970s and 1980s and have remained relatively unchanged since Harlow and Lane published their classic Antibodies: A Laboratory Manual in 1988.

Antibody Production (see page 5)

The term “antibody production” has both general and specific meanings. In the broad sense, it refers to the entire process of creating a usable specific antibody, including steps of immunogen preparation, immunization, hybridoma creation, collection, screening, isotyping, purification, and labeling for direct use in a particular method. The more restricted sense, antibody production refers to the steps leading up to antibody generation but does not include various forms of purifying and labeling the antibody for particular uses.

Antibody production involves preparation of antigen samples and their safe injection into laboratory or farm animals so as to evoke high expression levels of antigen-specific antibodies in the serum, which can then be recovered from the animal. Polyclonal antibodies are recovered directly from serum (bleeds). Monoclonal antibodies are produced by fusing antibody-secreting spleen cells from immunized mice with immortal myeloma cell to create monoclonal hybridoma cell lines that express the specific antibody in cell culture supernatant.

Successful antibody production depends upon careful planning and implementation with respect to several important steps and considerations:

- Synthesize or purify the target antigen (e.g., peptide or hapten)
- Choose an appropriate immunogenic carrier protein
- Conjugate the antigen and carrier protein to create the immunogen
- Immunize animals using appropriate schedule and adjuvant formula
- Screen serum (or hybridoma) for antibody titer and isotype (also called antibody characterization; see the following page)

Antibody Purification (see page 27)

Antibody purification involves isolation of antibody from serum (polyclonal antibody), ascites fluid or culture supernatant of a hybridoma cell line (monoclonal antibody). Purification methods range from very crude to highly specific:

- **Crude** – precipitation of a subset of total serum proteins that includes immunoglobulins
- **General** – affinity purification of certain antibody classes (e.g., IgG) without regard to antigen specificity
- **Specific** – affinity purification of only those antibodies in a sample that bind to a particular antigen molecule

Which level of purification is necessary to obtain usable antibody depends upon the intended application(s) for the antibody.

Antibody Characterization (see page 22)

Antibody characterization involves three kinds of activities that are usually performed in one form or another throughout an entire antibody production and purification project:

- Screen for antibody specificity and affinity
- Measure antibody concentration or titer
- Determine antibody isotype (class and subclass)

Screening is first required during production to identify which animals and hybridoma clones are producing a high level of antigen-specific antibody. This is usually accomplished using ELISA techniques.

Antibody concentration can be estimated using either a general protein assay or more species and immunoglobulin specific method, such as with specialized microagglutination assay kits. Antibody titer is related to concentration but refers more specifically to the effective potency of a given antibody sample. Measuring titer usually means determining the functional dilution of an antibody sample necessary for detection in a given assay, such as ELISA.

Isotyping involves determining the class (e.g., IgG vs. IgM) and subclass (e.g., IgG_1 vs. IgG_{2a}) of a purified monoclonal antibody. This is a critical step in antibody production, as it is necessary for choosing an appropriate purification and modification method for the molecule. Isotyping is most easily accomplished with commercial, ready-to-use antibody isotyping kits.

Antibody Fragmentation (see page 51)

Purified antibodies can be modified for particular uses by several methods including fragmentation into smaller antigen-binding units, conjugation with enzyme or other detectable markers, and immobilization to solid supports. Most often antibodies are used in whole-molecule form. However, the performance of some techniques and experiments can be improved by using antibodies whose nonessential portions have been removed.

Antibody Fragmentation refers to procedures for cleaving apart whole antibody molecules and removing portions that are not necessary for binding antigen. Fab and $F(ab)'_2$ are antibody fragments of IgG that are most frequently created and utilized by researchers.

Antibody Labeling and Immobilization (see page 61)

Antibodies are produced and purified for use as antigen-specific probes. However, their utility in any given technique (ELISA, Western blotting, cellular imaging, immunohistochemistry) depends upon having a mechanism to secondarily detect the antibody.

Techniques that utilize antibodies for immunoprecipitation or other form of affinity purification depend upon mechanisms for attaching or immobilizing them to chromatography media (e.g., beaded agarose resin). Strategies for accomplishing this involve the same considerations and chemical methods as antibody labeling.

References

Alberts, B., *et al.* (1983). *Molecular Biology of the Cell.* Garland Publishing, Inc., New York, NY.

Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sites, D.P., *et al.* (1976). *Basic & Clinical Immunology.* Lange Medical Publication, Los Altos, CA.

Structure of an Immunoglobulin Molecule

Antibody (or immunoglobulin) molecules are glycoproteins composed of one or more units, each containing four polypeptide chains: two identical heavy chains (H) and two identical light chains (L) (Figure 1). The amino terminal ends of the polypeptide chains show considerable variation in amino acid composition and are referred to as the variable (V) regions to distinguish them from the relatively constant (C) regions. Each L chain consists of one variable domain V_L and one constant domain C_L. The H chains consist of a variable domain, V_H, and three constant domains CH_1, CH_2 and CH_3. Each heavy chain has about twice the number of amino acids and MW (~50,000) as each light chain (~25,000), resulting in a total immunoglobulin MW of approximately 150,000.

Heavy and light chains are held together by a combination of noncovalent interactions and covalent interchain disulfide bonds, forming a bilaterally symmetric structure. The V regions of H and L chains comprise the antigen-binding sites of the immunoglobulin (Ig) molecules. Each Ig monomer contains two antigen-binding sites and is said to be bivalent.[1]

The hinge region is the area of the H chains between the first and second C region domains and is held together by disulfide bonds. This flexible hinge region allows the distance between the two antigen-binding sites to vary.[2]

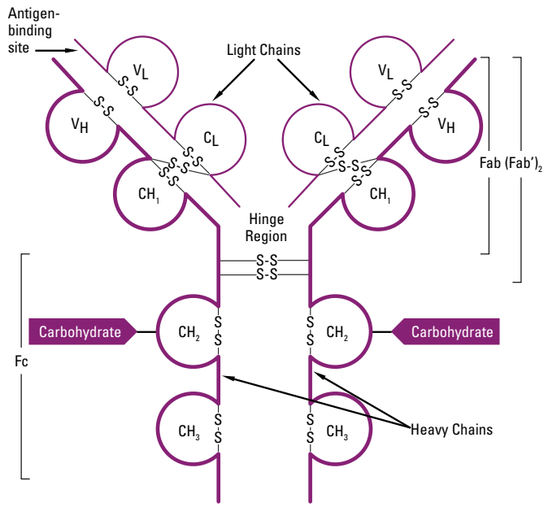


Figure 1. Structure of an immunoglobulin.

Immunoglobulin Classes and Subclasses

The five primary classes of immunoglobulins are IgG, IgM, IgA, IgD and IgE. These are distinguished by the type of heavy chain found in the molecule. IgG molecules have heavy chains known as γ-chains; IgMs have μ-chains; IgAs have α-chains; IgEs have ε-chains; and IgDs have δ-chains. Differences in heavy chain polypeptides allow these immunoglobulins to function in different types of immune responses and at particular stages of the immune response. The polypeptide protein sequences responsible for these differences are found primarily in the Fc fragment. While there are five different types of heavy chains, there are only two main types of light chains: kappa (κ) and lambda (λ).

Antibody classes differ in valency as a result of different numbers of Y-like units (monomers) that join to form the complete protein. For example, in humans, IgM antibodies have five Y-shaped units (pentamer) containing a total of 10 light chains, 10 heavy chains and 10 antigen-binding sites.

IgG, a monomer, is the predominant Ig class present in human serum. Produced as part of the secondary immune response to an antigen, this class of immunoglobulin constitutes approximately 75% of total serum Ig. IgG is the only class of Ig that can cross the placenta in humans, and it is largely responsible for protection of the newborn during the first months of life.[1] Because of its relative abundance and excellent specificity toward antigens, IgG is the principle antibody used in immunological research and clinical diagnostics.

Serum IgM exists as a pentamer in mammals, predominates in primary immune responses to most antigens, is the most efficient complement-fixing immunoglobulin and constitutes approximately 10% of normal human serum Ig content. IgM is also expressed on the plasma membrane of the B lymphocytes as a monomer. It is the B cell antigen receptor, and the H chains each contain an additional hydrophobic domain for anchoring in the membrane. Monomers of serum IgM are bound together by disulfide bonds and a joining (J) chain.

Each of the five monomers is composed of two light chains (either κ or λ) and two heavy chains. Unlike in IgG, the heavy chain in IgM is composed of one variable and four constant domains with no hinge region. IgM can cause cell agglutination as a result of recognition of epitopes on invading microorganisms. This Ab/Ag immune complex is then destroyed by complement fixation or receptor-mediated endocytosis by macrophages.

IgA exists in serum in both monomeric and dimeric forms, constituting approximately 15% of the total serum Ig. Secretory IgA, a dimer, provides the primary defense mechanism against some local infections because of its abundance in membrane secretions (e.g., saliva, tears). The principal function of secretory IgA may not be to destroy antigen, but to prevent passage of foreign substances into the circulatory system.

IgD and IgE are found in serum in much smaller quantities than other Igs. Membrane IgD is a receptor for antigen found mostly on mature B lymphocytes. IgE primarily defends against parasitic invasion.

In addition to the major immunoglobulin classes, several Ig subclasses based on minor differences in heavy chain type of each Ig class exist in all members of a particular animal species. In humans there are four subclasses of IgG: IgG_1, IgG_2, IgG_3 and IgG_4 (numbered in order of decreasing concentration in serum). Variance between different subclasses is less than the variance between different classes. For example, IgG_1 is more closely related to IgG_2, IgG_3 or IgG_4 than to IgA, IgM, IgD or IgE. Consequently, there is general cross-reactivity among subclasses but very little cross-reactivity among different classes of Ig.

Polyclonal and Monoclonal Antibodies

Antibodies (whatever their class or subclass) are produced and purified in two basic forms for use as reagents in immunoassays: polyclonal and monoclonal. Typically, the immunological response to an antigen is heterogeneous, resulting in many different cell lines of B lymphocytes (precursors of plasma cells) producing antibodies to the same antigen. All of these cells originate from common stem cells, yet each develops the individual capacity to make an antibody that recognizes a particular determinant (epitope) on the same antigen. As a consequence of this heterogeneous response, serum from an immunized animal will contain numerous antigen-specific antibody clones, potentially of several different Ig classes and subclasses comprising generally 2-5% of the total Ig. Because it contains this heterogeneous collection of antigen-binding immunoglobulins, an antibody purified from such a sample is called a polyclonal antibody. Polyclonal antibodies are especially useful as labeled secondary antibodies in immunoassays.

Since an individual B lymphocyte produces and secretes only one specific antibody molecule, clones of B lymphocytes produce monoclonal antibodies. All antibodies secreted by a B cell clone are identical, providing a source of homogeneous antibody having a single defined specificity. However, while B lymphocytes can be isolated from suspensions of spleen or lymph node cells excised from immunized animals, they have a limited life span and cannot be cultured directly to produce antibody in useful amounts.

Fortunately, this restriction has been overcome with the development of hybridoma technology, wherein isolated B lymphocytes in suspension are fused with myeloma cells from the same species (usually mouse) to create monoclonal hybrid cell lines that are virtually immortal while still retaining their antibody-producing abilities.[3] Such hybridomas may be stored frozen and cultured as needed to produce the specific monoclonal antibody. Monoclonal antibodies are especially useful as primary antibodies in applications that require single-epitope specificity and an unchanging supply over many years of use. Hybridoma clones may be grown in cell culture for collection of antibodies from the supernatant or grown in the peritoneal cavity of a mouse for collection from ascitic fluid.

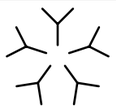
References

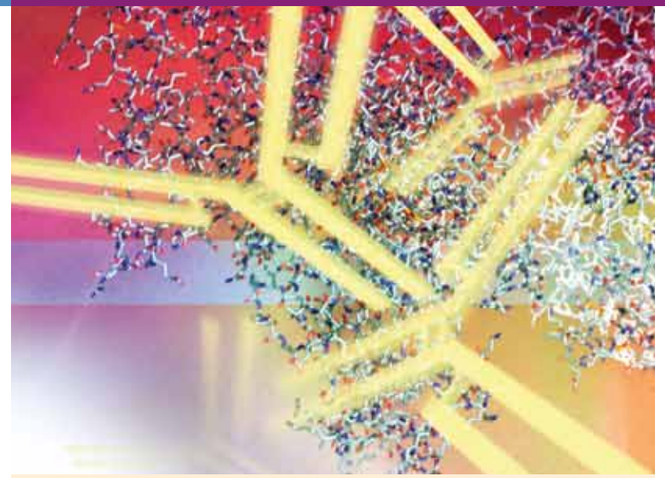
1. Sites, D.P., *et al.* (1976). *Basic & Clinical Immunology.* Lange Medical Publication, Los Altos, CA.
2. Alberts, B., *et al.* (1983). *Molecular Biology of the Cell.* Garland Publishing, Inc., New York, NY.
3. Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. (Product # 15050.)

Normal IgG concentrations from various sources.

Source	IgG concentration (approximate)
Ascites	2-10mg/mL
Serum	10-16mg/mL
Culture supernatant	0.5-1mg/mL

Important properties of antibody isotypes.

	IgG	IgM	IgA	IgE	IgD
Molecular weight	150,000	900,000	320,000 (secretory)		
Heavy chain: Type MW	γ 53,000	μ 65,000	α 55,000	ε 73,000	δ 70,000
Concentration in serum (approximate)	10-16mg/mL	0.5-2mg/mL	1-4mg/mL	0.00001-0.0004mg/mL	0-0.4mg/mL
Percent of total IgG	80	6	13	0.002	0.2
Carbohydrate (approximate)	3%	12%	10%	12%	13%
Distribution	Intravascular and extravascular	Mostly intravascular	Intravascular and secretions	Basophils and mast cells in saliva and nasal secretions	Lymphocyte surface
Function	Secondary response	Primary response	Protect mucous membranes	Protect against parasites	Unknown
Structure					



Antibody Production Overview

The production of specific antibody probes is a relatively straightforward process involving immunization of animals and reliance upon their immune systems to levy responses that result in biosynthesis of antibodies against the injected molecule. Even so, several factors affect the probability of inducing an immunized animal to produce useful amounts of target-specific antibodies. Antigens must be prepared and delivered in a form and manner that maximizes production of a specific immune response by the animal. This is called immunogen preparation.

Antibody production is conceptually simple. However, because it depends upon such a complex biological system (immunity of a living organism), results are not entirely predictable. Individual animals – even those that are genetically identical – will respond uniquely to the same immunization scheme, generating different suites of specific antibodies against an injected antigen. Even so, equipped with a basic understanding of how the immune system responds to injection of a foreign substance and a knowledge of available tools for preparing a sample for injection, researchers can greatly increase the probability of obtaining a useful antibody product.

For example, small compounds (drugs or peptides) are not sufficiently complex by themselves to induce an immune response or be processed in a manner that elicits production of specific antibodies. For antibody production to be successful with small antigens, they must be chemically conjugated with immunogenic carrier proteins such as keyhole limpet hemocyanin (KLH). Adjuvants can be mixed and injected with an immunogen to increase the intensity of the immune response.

Carrier protein conjugation, use of adjuvants and other issues relating to preparation of samples for injection are described in this section on antibody production. Procedures for generating, purifying and modifying antibodies for use as antigen-specific probes were developed during the 1970s and 1980s and have remained relatively unchanged since Harlow and Lane published their classic book *"Antibodies: A Laboratory Manual"* in 1988.

The Immune System

The immune system is a surveillance system designed to provide protection to its host from foreign invaders. The surveillance is mediated by proteins and cells that circulate throughout the organism to identify and destroy foreign cells, viruses or macromolecules.

Immune protection is provided by a dual system consisting of the cellular immune response and the humoral immune response. The cellular immune response is mediated by T lymphocytes and cannot be transferred from one individual to another by transfusion of serum. Humoral immunity involves soluble proteins found in serum (antibodies) that can be transferred to a recipient when serum is transfused.

Every cell in a vertebrate organism expresses the class I Major Histocompatibility Complex (MHC I) on its plasma membrane. The MHC I presents endogenously derived peptide antigens to cytotoxic T lymphocytes (CTL). If the T cell receptor of a CTL binds to the MHC I/peptide antigen on a cell, the whole cell is destroyed. This is a general description of the cellular immune response, which targets intracellular pathogens such as viruses or bacteria (non-self) and cancer cells (altered self).

The humoral response targets extracellular antigens. B-lymphocytes use membrane IgM (mIgM) to bind antigen in its native form. Crosslinking of many mIgM and antigen molecules occurs (capping) and the complex is then taken into the cell by receptor mediated endocytosis. This endosome then fuses with a lysosome and the resulting endolysosome digests the antigen into small peptides. The endolysosome fuses with a vesicle containing class II Major Histocompatibility Complex (MHC II) molecules and the peptide antigens are bound by a cleft in the MHC II. This MHC II/antigen complex is then expressed on the plasma membrane of the B-lymphocyte. The T cell receptor of a T helper lymphocyte then binds the MHC II/antigen and the T cell secretes cytokines that signal the B-lymphocyte to divide, differentiate and secrete antibodies. Without T help, the humoral response shuts down; in fact, the cellular response shuts down as well, as in AIDS.

Immunogenicity

Antigens and Immunogen

Successful generation of antibodies depends upon B-lymphocytes to bind, process and present antigen to T helper lymphocytes, which signal the B cells to produce and secrete antibodies. An antigen is any molecule that is identified as non-self by components of the immune system. An immunogen is an antigen that is able to evoke an immune response, including production of antibody via the humoral response. All immunogens are antigens, but not all antigens are immunogens. It is important to distinguish between the terms "antigen" and "immunogen" because many compounds are not immunogenic, and successful production of antibodies against such antigens requires that they be made immunogenic by chemically attaching them to known immunogens before injection.

Properties Determining Immunogenicity

Immunogenicity is the ability of a molecule to solicit an immune response. There are three characteristics that a substance must have to be immunogenic: foreignness, high molecular weight and chemical complexity. Foreignness is required so that the immunized animal does not recognize and ignore the substance as "self." Generally, compounds from an organism are not immunogenic to that same individual and are only poorly immunogenic to others of the same or related species.

The second requirement for immunogenicity is high molecular weight. Small compounds (MW less than 1000), such as penicillin, progesterone and aspirin, as well as many moderately sized molecules (MW from 1000 to 6000), are not immunogenic. Most compounds with a molecular weight greater than 6000 are immunogenic. Compounds smaller than this can often be bound by mIgM on the surface of the B-lymphocyte, but they are not large enough to facilitate crosslinking of the mIgM molecules. This crosslinking is commonly called "capping" and is the signal for receptor mediated endocytosis of the antigen.

Finally, some degree of chemical complexity is required for a compound to be immunogenic. For example, even high molecular weight homopolymers of amino acids and simple polysaccharides seldom make good immunogens because they lack the chemical complexity necessary to generate an immune response.

Macromolecules as Immunogens

It is possible to make certain generalizations about immunogenicity of the four major classes of macromolecules: carbohydrates, lipids, nucleic acids and proteins. Carbohydrates are immunogenic only if they have a relatively complex polysaccharide structure or form part of more complex molecules, such as glycoproteins. Lipids usually are not immunogenic but can be made so by conjugation to a carrier protein. Likewise, nucleic acids are poor immunogens but can become immunogenic when coupled to a carrier protein. Because of their structural complexity and size, proteins are generally strong immunogens. Given that most natural immunogens are macromolecules composed of protein, carbohydrate or a combination of the two, it is understandable that proteins are so broadly immunogenic. Peptides may have the complexity necessary to be antigenic, but their small size usually renders them ineffective as immunogens on their own. Peptides are most often conjugated to carrier proteins to insure that they induce an immune response and production of antibodies.

Haptens vs. Epitopes

Peptides and other small molecules that are used as antigens are referred to as haptens. They are able to act as recognition sites for production of specific antibodies but cannot by themselves stimulate the necessary immune response. Haptens can be made immunogenic by coupling them to a suitable carrier molecule.

An epitope is the specific site on an antigen to which an antibody binds. For very small antigens, practically the entire chemical structure may act as a single epitope. Depending on its complexity and size, an antigen may effect production of antibodies directed at numerous epitopes. Polyclonal antibodies are mixtures of serum immunoglobulins and collectively are likely to bind to multiple epitopes on the antigen. Monoclonal antibodies by definition contain only a single antibody clone and have binding specificity for one particular epitope.

Specific antibodies can be generated against nearly any sufficiently unique chemical structure, either natural or synthetic, as long as the compound is presented to the immune system in a form that is immunogenic. The resulting antibodies may bind to epitopes composed of entire molecules (e.g., small haptens), particular functional groups of a larger molecule, unique arrangements of several amino acid functional groups in the tertiary structure of proteins, or any other unique structure in lipoproteins, glycoproteins, RNA, DNA or polysaccharides. Epitopes may also be parts of cellular structures, bacteria, fungi or viruses.

Carrier Proteins Background

A carrier protein is any protein used for coupling with peptides or other haptens that are not sufficiently large or complex on their own to induce an immune response and produce antibodies. The carrier protein, because it is large and complex, confers immunogenicity to the conjugated hapten, resulting in antibodies being produced against epitopes on the hapten and carrier. Many proteins can be used as carriers and are chosen based on immunogenicity, solubility, and availability of useful functional groups through which conjugation with the hapten can be achieved. The two most commonly used carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA).

In a typical immune response, antibodies are produced by B-lymphocytes. In the majority of hapten-carrier systems, the B cells will produce antibodies that are specific for both the hapten and carrier. Because an antibody response will be directed against epitopes on both the carrier protein and hapten, it is important to plan carefully how hapten-specific antibodies will be identified and purified from the final immunized serum. To create the best immunogen, it may be beneficial to prepare the conjugates with several different carriers and with a range of hapten:carrier coupling ratios.

Keyhole Limpet Hemocyanin (KLH)

Keyhole limpet hemocyanin (KLH) is the most widely used carrier protein. The copper-containing polypeptide belongs to a group of non-heme proteins called hemocyanins, which are found in arthropods and mollusks. KLH is isolated from keyhole limpets *(Megathura crenulata)*.

Because KLH is from a class of proteins and a group of organisms that are evolutionarily distant from mammals, it is very "foreign" to the animal systems typically used to produce antibodies. The protein is also highly immunogenic because of its very large size and complex structure. The molecule is composed of 350kDa and 390kDa subunits that associate to form aggregates ranging from 0.5 to 8 million daltons.

Each KLH protein molecule contains several hundred surface lysine groups that provide primary amines as targets for covalent attachment of haptens using a variety of crosslinking techniques. These features make KLH an extremely immunogenic and effective carrier protein for immunogen preparation. Although the large protein is sometimes difficult to work with because it has limited solubility, the commercial availability of stabilized and pre-activated formulations make it convenient to use.

Thermo Scientific Imject Mariculture Keyhole Limpet Hemocyanin (mcKLH) is purified and lyophilized in a stabilizing buffer. After reconstitution, the suspension-solution is an opalescent blue, which is characteristic of highly purified, nondenatured KLH.

Traditionally, KLH was obtained from giant keyhole limpets harvested directly from the natural environment. This method disturbs the sensitive shoreline ecosystems where these limpets live. Current methods to obtain KLH are much less threatening to the natural habitat and limpet species survival. Giant keyhole limpets are raised in tanks and harvested (marine culture or "mariculture") where they are occasionally milked of some of their fluids, similar to humans donating blood.

PEGylated Keyhole Limpet Hemocyanin (PEG KLH)

During antigen conjugation to carrier proteins, precipitation can lead to loss of antigen and reduced immune response. Since most carrier proteins have extremely high molecular weight, insolubility can result in loss of antigen and sub-optimal immunization efficiency. To overcome difficult in handling, we have derivatized mcKLH with maleimide groups possessing a polyethylene glycol spacer arm (PEG spacer) (Figures 1 and 2). Poly(ethylene glycol) has several chemical properties that make it especially useful in various biological, chemical and pharmaceutical settings:

- **Non-toxic and non-immunogenic** – can be added to media and attached to surfaces and conjugated to molecules without interfering with cellular functions or target immunogenicities
- **Hydrophilic (aqueous-soluble)** – attachment to proteins and other biomolecules decreases aggregation and increases solubility
- **Highly flexible** – provides for surface treatment or bioconjugation without steric hindrance

PEGylated mcKLH is the first KLH to offer enhanced water solubility (150mg/mL). Additionally, PEG is less immunogenic than traditional chemical crosslinkers which have a hydrocarbon spacer arm. This reduces the threat of antibody production targeting the chemical spacer arm between the carrier protein and antigen.

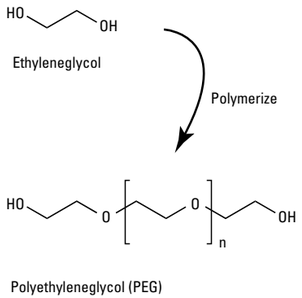


Figure 1. Ethylene glycol polymerization.

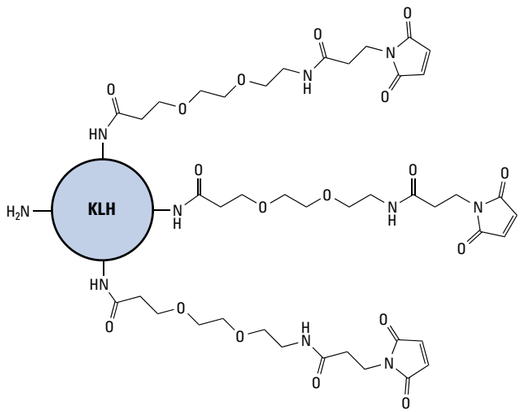


Figure 2. KLH derivitized with maleimide functional groups possessing a PEG spacer.

Blue Carrier Immunogenic Protein

Blue Carrier Protein is a purified preparation of *Concholepas concholepas* hemocyanin (CCH). The large protein exhibits most of the same immunogenic properties as the popular carrier protein, keyhole limpet hemocyanin (KLH). However, its significantly better solubility provides greater flexibility in immunogen preparation protocols by allowing a broader range of buffer and pH conditions for coupling peptides, proteins and other haptens using crosslinking methods.

Blue Carrier Protein is specially purified hemocyanin from the mollusk *Concholepas concholepas*. The CCH protein is composed of two very large polypeptide subunits (404 and 351kDa) that form an extremely stable heterodidecameric structure even in the absence of divalent cations. (By contrast, KLH has a less stable and soluble homodidecameric structure). The complex molecular arrangement of CCH subunits contains diverse repeated antigenic structures that elicit a strong immune reaction mediated by T and B lymphocytes.

Because of their large size and molecular complexity, KLH and CCH hemocyanins are carrier proteins of choice for use as immunogens to produce antibodies against haptens and peptides. Moreover, studies suggest that the strong DTH immune response elicited by hemocyanins in animals and in humans has beneficial therapeutic effects in certain types of cancer. New developments in the immunotherapy of cancer have taken advantage of the unique immunogenic properties of hemocyanins in the development of novel conjugate vaccines for treatment of emerging diseases.

Bovine Serum Albumin

Bovine serum albumin (BSA; 67kDa) belongs to the class of serum proteins called albumins. Albumins constitute about half the protein content of plasma and are quite stable and soluble. BSA is much smaller than KLH but is nonetheless fully immunogenic. It is a popular carrier protein for weakly antigenic compounds. BSA exists as a single polypeptide with 59 lysine residues, 30 to 35 of which have primary amines that are capable of reacting with a conjugation reagent. Numerous carboxylate groups give BSA its net negative charge (pI 5.1). Thermo Scientific Imject BSA is a highly purified (i.e., Fraction V) bovine serum albumin that, once reconstituted, can be used for conjugation to haptens without dialysis or further purification.

BSA is commonly used in development of immunoassays because it is readily available, is fully soluble and has numerous functional groups useful for crosslinking to small molecules that otherwise would not coat efficiently in polystyrene microplates. Furthermore, BSA is the most popular standard for protein assays, well-characterized as a molecular weight marker in SDS-PAGE and widely used as a blocking agent. These same characteristics that make BSA easy to use in immunoassay development also make it simple to use for preparing and testing conjugation efficiency of carrier-hapten conjugates. However, such multiple uses for BSA also require that steps be taken to avoid undesired cross-reactivity with the carrier in antibody-screening procedures and final applications.

For this reason, BSA is often used as a non-relevant protein carrier for antibody screening and immunoassays after using KLH as the carrier protein to generate the immune response against the hapten. Only by using different carrier proteins in the immunization and screening/purification steps can one be assured of detecting hapten-specific rather than carrier-specific antibodies. Using BSA as the non-relevant carrier protein generally allows one to take greater advantage of its properties as standard, MW marker and blocking agent.

Cationized BSA

Cationized bovine serum albumin (cBSA) is prepared by modifying native BSA with excess ethylenediamine, essentially capping all negatively-charged carboxyl groups with positively-charged primary amines (Figure 3). The result is a highly positively-charged protein (pI > 11) that has significantly increased immunogenicity compared to native BSA. In addition, the increased number of primary amines provides for a greater number of antigen molecules to be conjugated with typical crosslinking methods.

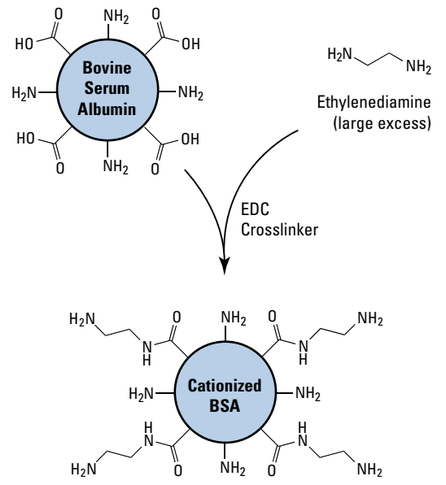


Figure 3. Preparation of cationized BSA. Bovine serum albumin is reacted with an excess of ethylenediamine using EDC.

A series of research articles by Muckerheide, Domen and Apple (1987, 1988) reported these researchers' studies of the effects of carrier protein cationization on the generation and regulation of immune responses. In their studies, using cBSA as the carrier protein resulted in immunogen that stimulated a much higher antibody response than the native form of BSA. *In vivo*, the antibody response increased and remained elevated for an extended period of time. *In vitro*, much less cBSA than native BSA was required to produce the same degree of T cell proliferation. Interestingly, the immune response enhancement caused by cBSA extended to haptens or other proteins to which it was conjugated. For example, when used to immunize mice, ovalbumin conjugated to cBSA elicited greater anti-ovalbumin antibody production than ovalbumin alone or ovalbumin-BSA conjugate.

Ovalbumin

Ovalbumin (OVA; 45kDa) can be used as a carrier protein. Also known as egg albumin, ovalbumin constitutes 75% of protein in hen egg whites. OVA contains 20 lysine groups and is most often used as a secondary (screening) carrier rather than for immunization, although it is somewhat immunogenic. The protein also contains 14 aspartic acid and 33 glutamic acid residues that afford carboxyl groups. These groups can be used as targets for conjugation with haptens. Ovalbumin exists as a single polypeptide chain having many hydrophobic residues and an isoelectric point of 4.63. The protein denatures at temperatures above 56°C or when subject to electric current or vigorous shaking. OVA is unusual among proteins in being soluble in high concentrations of the organic solvent DMSO, enabling conjugation to haptens that are not easily soluble in aqueous buffers.

Carrier Protein:Antigen Conjugation Methods

Methods of Hapten-Carrier Conjugation

Several approaches are available for conjugating haptens to carrier proteins. The choice of which conjugation chemistry to use depends on the functional groups available on the hapten, the required hapten orientation and distance from the carrier, and the possible effect of conjugation on biological and antigenic properties. For example, proteins and peptides have primary amines (the N-terminus and the side chain of lysine residues), carboxylic groups (C-terminus or the side chain of aspartic acid and glutamic acid), and sulfhydryls (side chain of cysteine residues) that can be targeted for conjugation. Generally, it is the many primary amines in a carrier protein that are used to couple haptens via a crosslinking reagent.

EDC Conjugation (carboxyl and amine crosslinking)

Because most proteins contain both exposed lysines and carboxyl groups, immunogen formation with the carbodiimide crosslinker EDC is often the simplest and most effective method for protein-carrier and peptide-carrier conjugations. EDC reacts with available carboxyl groups on either the protein carrier or peptide hapten to form an active *o*-acylisourea intermediate. This intermediate then reacts with a primary amine to form an amide bond and a soluble urea by-product (Figure 4). This efficient reaction produces a conjugated immunogen in less than two hours.

In general, conjugations mediated by EDC result in a certain amount of polymerization when polypeptide antigens and protein carriers are involved. This occurs because most peptides and antigens contain both primary amines and carboxylates (at least in their N- and C-termini, respectively). Some peptides will conjugate to themselves (end-to-end by their N- and C-termini or through side chains) as well as to the carrier protein. Likewise, the carrier protein will conjugate to itself.

Such polymerization is not necessarily a disadvantage for immunogenicity or the desired antigen-specific antibody production. Large polymers can decrease the solubility of the conjugate, making its subsequent handling and use more difficult. Some polymerized peptide on the surface of the carrier may actually enhance the immunogenicity of the peptide, effecting a greater antibody response. Peptides will become conjugated in a variety of orientations, ensuring that all parts of the molecule are presented and available as antigens within the entire population.

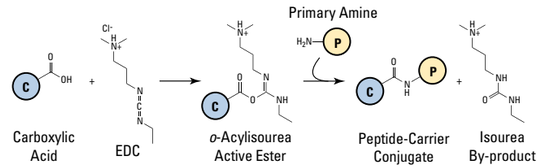


Figure 4. EDC mediated conjugation of peptides and carrier proteins. Carrier proteins (C) and peptides (P) have both carboxyls and amines, so conjugation occurs in both orientations. Carrier proteins are very large in comparison to typical peptide haptens; therefore, numerous conjugation sites exist on each carrier protein molecule.

Maleimide Conjugation (sulfhydryl crosslinking)

A peptide synthesized with a terminal cysteine residue has a sulfhydryl group that provides a highly specific conjugation site for reacting with certain crosslinkers. For example, the heterobifunctional crosslinker Sulfo-SMCC contains a maleimide group that will react with free sulfhydryls, plus and a succinimidyl (NHS) ester that will react with primary amines. By reacting the reagent first to the carrier protein (with its numerous amines) and then to a peptide containing a reduced terminal cysteine, all peptide molecules can be conjugated with the same predictable orientation.

It is a two-step reaction strategy (Figure 5). The carrier protein is "activated" in isolation by reaction with a molar excess of Sulfo-SMCC. Numerous molecules of SMCC become attached to the carrier protein when the NHS-ester group is displaced by the abundant amino group of the carrier protein. The modified carrier protein is then purified by gel filtration (desalting) to remove excess crosslinker and byproducts. At this stage, the purified carrier possesses modifications generated by the crosslinker, resulting in a number of reactive maleimide groups projecting from its surface. Finally, cysteine-terminated peptide or other sulfhydryl hapten is added to the maleimide-activated carrier protein. The maleimide groups react with the peptide sulfhydryl (–SH) groups to form stable thioether bonds.

Any protein can be maleimide-activated in this manner to allow efficient conjugation of haptens via reduced thiols. However, KLH, BSA and other popular carrier proteins are available in pre-activated forms and convenient kits that are ready for immediate conjugation with sulfhydryl peptides. Purchasing quality-tested, stabilized, maleimide-activated carrier proteins ensures consistent performance and saves several steps.

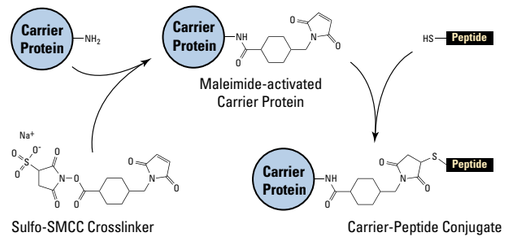


Figure 5. Maleimide activation and carrier-peptide conjugation with Sulfo-SMCC crosslinker. Carrier proteins possess numerous (tens to hundreds) of primary amines per molecule. Therefore, each carrier protein molecule receives many maleimide activations and can conjugate many peptide haptens.

Glutaraldehyde Conjugation (amine-to-amine crosslinking)

Glutaraldehyde can be used to crosslink peptides and carrier proteins via amines on the respective polypeptides. This approach randomly targets lysine residues or the N-terminus of a peptide and surface lysines of the carrier protein (Figure 6). Depending upon the peptide amino acid composition (i.e., whether it possesses more than one primary amine), the opportunity for variable antigen presentation (orientation) and high loading (polymerization) are not as great as with EDC conjugation. However, glutaraldehyde is an efficient crosslinker, if not particularly specific and predict-able, and it is still commonly used by antibody production facilities.

Amine-to-amine crosslinking also can be accomplished with crosslinkers such as disuccinimidyl suberate (DSS) and its water-soluble analog, BS3. If longer spacer arms are desired, pegylated versions of BS3 are also available.

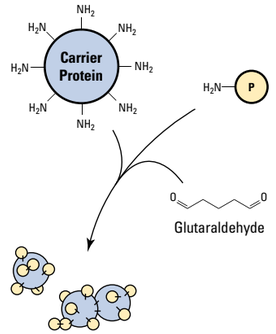


Figure 6. Glutaraldehyde crosslinking between carrier protein and antigen.

Other Hapten-carrier Conjugation Chemistries

Haptens, including many drugs, steroids and polysaccharides, do not contain amines, carboxylates or sulfhydryls. Yet, conjugation to a carrier protein is necessary if they are to be made immunogenic and allow production of antibody.

In theory, any of our crosslinking and modification reagents can be used to prepare hapten-carrier protein conjugates. For example, haptens containing sugar groups or polysaccharide chains can be conjugated by reductive amination to primary amines on carrier proteins. The reaction requires that diols in sugar rings be oxidized to active aldehydes, which will react to primary amines.

Any molecule that has an active hydrogen can be conjugated to primary amines in the presence of formaldehyde, a scheme known as the Mannich Reaction (Figure 7).

When choosing a conjugation chemistry for preparation of an immunogen, care must be taken to prevent altering the hapten too much or the antibodies raised against its epitopes will not recognize the native target molecule. For peptides, careful attention to their amino acid composition and sequence is necessary (presence of residues containing amines, carboxylates and cysteines). For polysaccharides, the effects of oxidation on their overall structure must be considered. Synthetic design of the peptides and other haptens allows for the addition of unique functional groups that can be used for conjugation without affecting the intended epitopes.

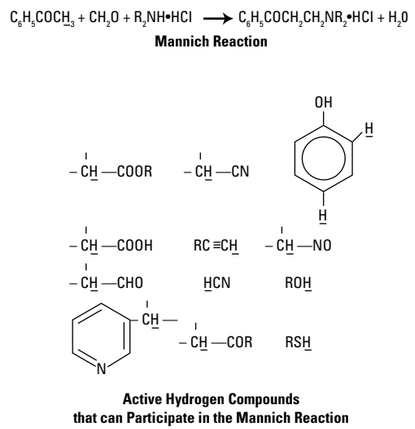


Figure 7. Examples of Mannich Reaction and active hydrogen compounds in Mannich Reaction.

Comparison of Carrier Proteins

While each carrier protein possesses specific differentiating traits, they are rarely compared across the same parameters. Below is a study we performed to evaluate the various carrier protein options along the characteristics of solubility, activation level for sulfhydryl containing antigen conjugation, peptide coupling efficiency, purification after peptide coupling, and finally the ability to elicit an immune response.

Solubility

Solubility is an important consideration with regard to the use of carrier proteins. KLH is the most popular and highly immunogenic carrier protein, but it is also known for having poor solubility. This can make it difficult to prepare for crosslinking reactions and to recover conjugate in subsequent clean-up steps.

Our data indicate that the various forms of Imject mcKLH and related Blue Carrier Protein products have more than sufficient solubility for use in immunogen preparation and subsequent immunization protocols (Figure 8). Maleimide activation decreases the solubility of a given protein type, but the effect on KLH is offset by modification with polyethylene glycol (PEG). Blue Carrier Protein is more soluble than mcKLH, especially when maleimide-activated forms are compared.

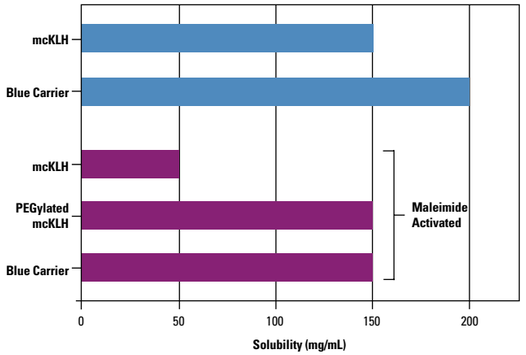


Figure 8. Thermo Scientific Blue Carrier Protein and PEGylated mcKLH have improved solubility over mcKLH. Blue Carrier Protein is supplied as a solution in PBS at 200mg/mL. mcKLH is supplied as a solid lyophilized from a PBS solution. A 150mg/mL solution of mcKLH is achievable which is 50mg/mL less soluble than Blue Carrier Protein. A comparison of the maximum solubility of three lyophilized maleimide activated carrier proteins shows maleimide KLH to be the least soluble. The maximum concentration for Maleimide mcKLH is 50mg/mL and this solution is also slightly cloudy. The addition of PEGylated spacer the maleimide mcKLH results in a three times more soluble (150mg/mL) solution than maleimide mcKLH. Maleimide Blue Carrier Protein is also three times more soluble than maleimide mcKLH.

Levels and Quality of Maleimide Activation

Maleimide activation of carrier proteins makes it possible to conjugate sulfhydryl-containing haptens. For a number of reasons, this is an especially effective and popular strategy for conjugating cysteine-terminated peptide haptens. Maleimide activation can be accomplished using Thermo Scientific Pierce Sulfo-SMCC crosslinker (or similar reagent), but it is difficult to obtain and validate consistently high levels of activation. Our Imject Maleimide-Activated Carrier Proteins are prepared using a very reliable procedure, and each lot is tested to confirm that a high level of activation was achieved.

We use a reliable cysteine coupling assay to determine the moles of maleimide per moles carrier protein. Briefly, activated carrier proteins are dissolved in water at 10mg/mL and then diluted to 0.5mg/mL in Assay Buffer (0.1M sodium phosphate, 0.1M EDTA, pH 7.2). The diluted activated carrier proteins are transferred to a microplate (200µL/well). A dilution series of L-cysteine is prepared (1mg/mL to 0.25mg/mL) in Assay Buffer and 10µL of each dilution is added to a well containing protein solution. For a blank control, Assay Buffer is added to the test sample. The maleimide activated carrier protein is allowed to react with the cysteine for 2 hours at room temperature. Finally, the amount of noncoupled cysteine is determined using Thermo Scientific Ellman's Reagent (5,5'-Dithio-bis-[2-nitrobenzoic acid]). Samples are incubated for 15 minutes with 20µL of a 6.3mM Ellman's Reagent prepared in Assay Buffer, after which the absorbance is measured at 405nm. The proportion (and absolute moles) of cysteine coupled is calculated by comparing the absorbance of the maleimide activated carrier samples to the absorbance of nonactivated carrier protein controls (Figure 9).

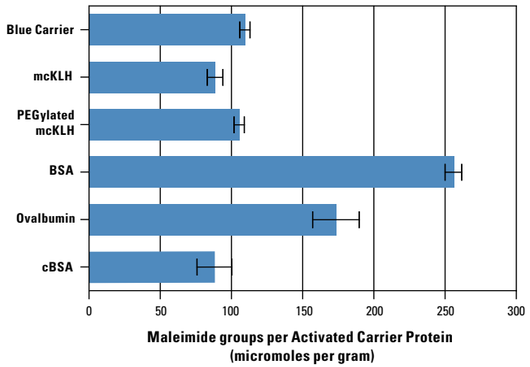


Figure 9. High levels of maleimide activation of Thermo Scientific Imject Carrier Proteins. Surface primary amines (i.e., lysine side chains) of Imject Carrier Proteins are rendered sulfhydryl-reactive by reaction with Thermo Scientific Pierce Sulfo-SMCC crosslinker. They contain a molar excess of maleimide for coupling cysteine containing haptens (peptide or proteins). Activation levels (moles of maleimide per gram of carrier protein) were determined with a cysteine coupling assay. KLH and Blue Carrier Proteins are very large (approx. 8000kDa), enabling activation with 600 to 900 maleimide groups per protein molecule. BSA and ovalbumin are smaller (67kDa and 45kDa, respectively), allowing for activation with 5 to 20 maleimide groups per protein molecule.

Peptide Coupling Efficiency

A key practical feature of any useful carrier protein is its ability to efficiently conjugate with haptens, especially peptides of various kinds. Thus, we compared our various carrier protein products for their ability to conjugate two peptides having very different chemical properties (hydrophilic vs. hydrophobic).

We tested two fluor-labeled peptides:

- CaM Kinase II Substrate 281-291, 5-FAM labeled (5-FAM-MHRQETVDCLK-NH2, hydrophilic, Anaspec). Reconstituted in ultrapure water for use
- EMP17, FITC-LC labeled (FITC-LC-TYSCHFGPLTWVCKPQGG-OH, hydrophobic, Anaspec). Reconstituted in DMSO for use

For cysteine coupling, peptides were reconstituted at 10mg/mL, then 0.3 to 0.4mg of peptide was combined with 1mg of maleimide-activated carrier protein and conjugation buffer (Product # 77164) for a total reaction volume of 300µL. For EMP17 peptide DMSO was added to a final concentration of 30% in the reaction and DTT added to a final molarity of 1mM. Reactions were incubated for 2 hours at room temperature. The conjugates were desalted to remove uncoupled peptide and EDTA.

For coupling with EDC, peptides were reconstituted at 20mg/mL. Carrier proteins were dissolved at 10mg/mL in ultrapure water. EDC was dissolved at 10mg/mL in ultrapure water immediately before addition. Then, 0.8mg peptide was combined with 1mg carrier protein and the volume adjusted to 300µL with EDC conjugation buffer (Product # 77162). For EMP17 peptide, DMSO was added to a final concentration of 30%. Finally, 25µL of EDC solution was added and the reaction was incubated for 2 hours at room temperature. The reaction was then desalted into Imject Purification Buffer (Product # 77159).

Conjugation efficiency was determined by measuring the relative fluorescent units (RFU) of the conjugates on compatible fluorescent plate reader (Figure 10). The RFU was compared to a standard of unconjugated peptides. Results were normalized to the mcKLH-peptide conjugates.

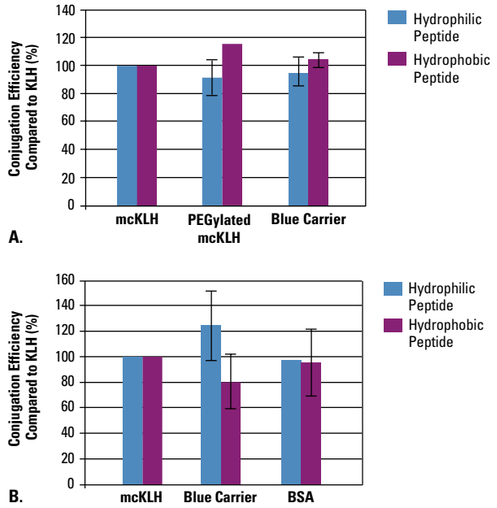


Figure 10. Carrier proteins effectively conjugate hydrophilic and hydrophobic peptide through cysteines and amines. Two fluorescently labeled peptides (5-FAM-MHRQETVDCLK-NH2 (hydrophilic), and FITC-LC-TYSCHFGPLT-WVCKPQGG-OH (hydrophobic)) were coupled to mcKLH, PEGylated mcKLH Blue Carrier Protein and BSA. **(A)** Maleimide Blue Carrier Protein and Maleimide PEGylated KLH have slightly better peptide coupling efficiency through cysteines than maleimide mcKLH (n=5 for PEGylated KLH and Blue Carrier Protein, n=4 for KLH). PEGylated mcKLH performs the best for coupling hydrophobic peptide. **(B)** When coupling through amines using EDC chemistry all proteins (KLH, Blue Carrier, and BSA) perform with similar efficiency (n=6 for KLH and Blue Carrier, n=3 for BSA). When coupling hydrophilic peptides Blue Carrier Protein performs better than mcKLH and BSA.

Hapten:Carrier Protein Conjugate Recovery

Typically, hapten-carrier conjugates must be filtered or otherwise "cleaned up" following crosslinking reactions to remove excess crosslinker and exchange buffer components before using the prepared immunogen for immunization. Usually this is accomplished by gel filtration, i.e., a desalting column. Traditionally, and in the former versions of our Imject Kits, gravity-flow desalting columns were used for this purpose. Our updated kits use much faster, simpler, and convenient centrifuge desalting columns: Thermo Scientific Zeba Spin Desalting Columns, 7K MWCO.

To illustrate the advantages of this newer desalting technology, we compared results obtained with samples processed using both traditional drip columns (Thermo Scientific Pierce Dextran Desalting Columns) and the newer Zeba Columns (Figure 11). Peptide conjugate samples were desalted using columns equilibrated in Imject Purification Buffer (0.083M sodium phosphate, 0.9M sodium chloride, 0.1M sorbitol, pH 7.2). The Dextran Columns were equilibrated with 3 bed-volumes of buffer. The Zeba Columns were equilibrated by centrifugation with 3 x 1mL of buffer. Samples loaded on the Dextran Columns were desalted by gravity flow with 3 x 0.5mL aliquots of buffer; conjugates were recovered in about 1mL volume. Peptide-carrier conjugates were applied to the Zeba Spin Desalting columns, followed by a 50µL chase of buffer. The columns were centrifuged for 2 minutes at 1000 x *g*. Peptide-carrier conjugates were recovered in 350µL.

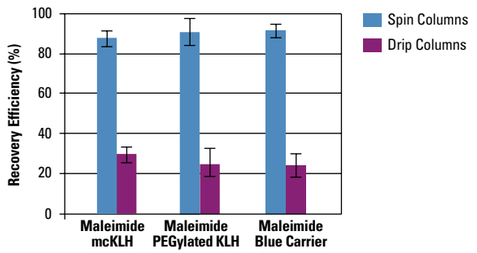


Figure 11. Thermo Scientific Zeba Spin Desalting Columns have improved recovery of carrier-peptide conjugates over Dextran Desalting Columns. Spin Kits provide improved sample recovery of the carrier-hapten conjugate. Columns were loaded with 300µL of sample (carrier-peptide conjugate). The sample was recovered in 350µL volume using the Zeba Desalt Spin Columns and in 1,000µL when using the Dextran Desalt Column (n=3). The dextran desalt columns and the Zeba Desalt Spin Columns recover similar amounts of carrier-hapten conjugate; however, the Zeba Desalt Column recovered the conjugate in less volume. The Zeba Spin Desalt Columns in the Carrier Conjugation kits provide excellent conjugate recovery in without diluting the sample.

Conjugate Immunogenecity and Antibody Production

Ultimately, the goal of immunogen preparation is to produce an immune response and concomitant production of hapten-specific antibodies by the immunized host animal. KLH is widely recognized as the benchmark or standard of immunogenicity: if another carrier protein or a variant of KLH elicits an immune response similar to standard KLH, it is a very good immunogen.

To compare and illustrate the immunogenic quality of our Imject PEGylated Maleimide Activated mcKLH and Blue Carrier Protein, we performed parallel immunogen preparation, immunization and antibody titer experiments with these products (Figure 12). Rabbits (n=2) were immunized with a synthetic peptide (QVPRRMIGTDAC) coupled to each carrier protein by three different methods. Immunizations were performed by Thermo Scientific Open Biosystems (custom polyclonal antibody production service). Collected sera (pre-immune and 35-day post-immunization) were screened by ELISA for peptide-specific antibody.

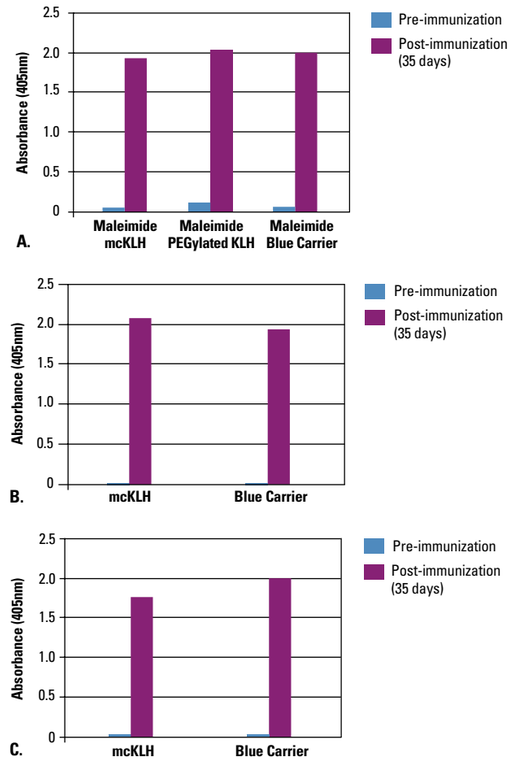


Figure 12. Thermo Scientific Carrier Proteins elicit a strong immune response to generate high levels of target antibody. Rabbits (n=2) were immunized with a synthetic peptide coupled to **(A)** maleimide-activated KLH, maleimide activated PEGylated mcKLH, and maleimide activated Blue Carrier Protein. Rabbit; **(B)** mcKLH and Blue Carrier Protein via glutaraldehyde coupling; **(C)** mcKLH and Blue Carrier Protein via EDC crosslinking. Rabbit serum samples were collected immediately before immunization and 35 days post-immunization. Serum samples were diluted and screened in an ELISA with the immunizing peptide coated directly onto the ELISA plate. ABTS was used as the substrate and measured at 405nm, which indicates the presence of an antibody specific for the immunizing peptide. Blue Carrier Protein, KLH and PEGylated KLH each elicited a similar immune response.

Choose the Right Carrier Protein

Delivering a large dose of antigen:carrier protein complex which will not be quickly degraded is essential for boosting the immune response. A carrier protein's massive size acts a scaffold which is carpeted with a peptide antigen. The peptide:carrier protein complex is shuttled throughout the bloodstream of a host, presenting the antigen to lymphocytes.

For initiating an immune response in a host organism, KLH and Blue Carrier are the most commonly used carrier proteins. Derived from sea mullosks/urchins, they help stimulate the mammalian immune response because the two species share little similarity. Remember that the host organism will produce antibodies against your peptide of interest as well as the carrier protein. Alternative carrier proteins such as BSA or Ovalbumin are commonly used during screening of sera as well as affinity purification to prevent detection of antibodies against KLH or Blue Carrier. For more on each carrier protein, see Table 1.

Table 1. Properties of Thermo Scientific Carrier Proteins.

	KLH	PEGylated KLH	Blue Carrier	BSA	Cationized BSA	Ovalbumin
Source	Mullosc, *Megathura Crenulata*	Mullosc, *Megathura Crenulata*	Mollusc, *Conchelepas conhelepas*	Bovine	Bovine	Chicken
Size	4.5 X 10^5 - 8.0 x 10^6 Daltons	4.5 X 10^5 - 8.0 x 10^6 Daltons	8.0 x 10^6 Daltons	6.7 x 10^6 Daltons	6.7 x 10^6 Daltons	4.5 x 10^6 Daltons
Solubility	50mg/mL	150mg/mL	200mg/mL	> 10mg/mL	> 10mg/mL	> 10mg/mL
Reactive towards reduced Sulfhydryls (-SH)	Maleimide activated version available	Maleimide activated version available	Maleimide activated version available	Maleimide activated version available	Maleimide activated version available	Maleimide activated version available
Reactive towards Carboxy or amine termini?	Yes, use EDC. Also available as pre-packaged kits to include EDC and optimized protocol.	Not available	Yes, use EDC. Also available as pre-packaged kits to include EDC and optimized protocol.	Yes, use EDC	Yes, use EDC	Yes, use EDC
Reactive towards primary amines	Yes, crosslink with Glutaraldehyde	Yes, crosslink with Glutaraldehyde	Yes, crosslink with Glutaraldehyde	Yes, crosslink with Glutaraldehyde	Yes, crosslink with Glutaraldehyde	Yes, crosslink with Glutaraldehyde
Common use	Primary immunization	Primary immunization	Primary immunization	Primary immunization	Secondary screen	Secondary screen

When looking for the right carrier protein for your research, consider the extensive line of Thermo Scientific Carrier Proteins.

Imject® Mariculture Keyhole Limpet Hemocyanin (mcKLH)

mcKLH is the most commonly used carrier protein for initiating an immune response from antibody producing animals. mcKLH is available underivitized for use in reactions involving EDC to crosslink primary amines (from lysine residues or the N-terminus) to carboxylic acids (from aspartic acid / glutamic acid residues or the C-terminus). Additionally, amine to amine crosslinlking between mcKLH and an antigen is possible using glutaraldehyde. mcKLH is available as pure protein in single use packages, large volume packages and optimized spin-kits which possess all of the components to facilitate carrier protein:antigen conjugate formation and clean-up.

Highlights:

- **Optimal** – formulated for enhanced solubility and stability
- **Active** – elicits a stronger immune response than BSA or OVA
- **Flexible** – lyophilized in PBS or MES buffer
- **Large** – high molecular mass (0.5 to 8.0 x 10^6 Da) for numerous conjugation sites
- **Eco-friendly** – cultivated from an environmentally friendly source

Ordering Information

Product #	Description	Pkg. Size
77600	**Imject mcKLH (in PBS)** Formulation: Lyophilized from 10mg/mL protein solution in phosphate-buffered saline with stabilizer, pH 7.2	5 x 20mg
77649	**Imject mcKLH Subunits, High Purity Research Grade** Formulation: Pure protein in 1mL sterile water.	20mg
77653	**Imject mcKLH (in MES Buffer)** Formulation: Lyophilized from 10mg/mL protein solution in 50mM MES, 150mM NaCl, 50mM sucrose, pH 6.5	2mg
77671	**Imject EDC mcKLH Spin Kit** *Sufficient for five conjugation reactions.* Kit Contents: Imject Mariculture KLH (in MES buffer) Imject EDC Conjugation Buffer EDC Crosslinker Imject Purification Buffer Salts Zeba Spin Desalting Columns, 7K MWCO	Kit 5 x 2mg 30mL 5 x 10mg 5 x 0.84g 5 x 2mL
77159	**Imject Purification Buffer Salts**	5g
77162	**Imject EDC Conjugation Buffer**	30mL

References
Cen, O., *et al.* (2003). *J. Biol. Chem.* **278**, 8837-8845.
Herreman, A., *et al.* (2003). *J. Cell Sci.* **116**, 1127-1136.
Jerry, D.J. (1993). *BioTechniques* **14(3)**, 464-469.

Imject® Maleimide Activated mcKLH and PEGylated mcKLH

To target reduced sulfhydryl groups from cysteine residues on your antigen, use maleimide activated mcKLH. Derivitazed with the crosslinker Sulfo-SMCC, maleimide activated mcKLH is optimized for the highest conjugation efficiency. Maleimide activated mcKLH is also available in a PEGylated form, which imparts superior solubility for easy handling during conjugation. Higher solubility enhances antigen conjugation efficiency and minimizes carrier protein:antigen conjugate loss. PEGylated mcKLH also reduces the chance of antibodies produced against the crosslinker spacer arm of Sulfo-SMCC.

Highlights:

- **Optimized** – achieve the highest antigen conjugation efficiency
- **Targeted** – cysteine reactive chemistry enables controlled orientation of your antigen
- **Flexible** – available in various package sizes
- **Easy to handle** – high solubility to minimize loss during conjugation

Ordering Information

Product #	Description	Pkg. Size
77605	**Imject Maleimide-Activated mcKLH** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	10mg
77606	**Imject Maleimide-Activated mcKLH** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	2mg
77610	**Imject Maleimide-Activated mcKLH** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	10 x 10mg
77663	**Imject Maleimide PEGylated mcKLH** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	10mg
77666	**Imject Maleimide-Activated mcKLH Spin Kit** *Sufficient for five conjugation reactions.* Kit Contents: Imject Maleimide-Activated mcKLH Imject Maleimide Conjugation Buffer Imject Purification Buffer Salts Zeba Spin Desalting Columns, 7K MWCO	Kit 5 x 2mg 30mL 5 x 0.84g 5 x 2mL
77164	**Imject Maleimide Conjugation Buffer**	30mL

Blue Carrier Immunogenic Protein

A new alternative to KLH, Blue Carrier protein is a more soluble and less expensive carrier protein. Derived from the organism *Conchelepas conchelepas,* Blue Carrier's size and species of origin make it a great immune response stimulator. Enhanced water solubility lend to easier handling during conjugation of carrier protein to antigen and higher stability. Blue Carrier is available underivitized for use in reactions involving EDC to crosslink primary amines (from Lysine residues or the N-terminus) to carboxylic acids (from aspartic acid / glutamic acid residues or the c-terminus). Additionally, amine to amine crosslinlking between mcKLH and an antigen is possible using glutaraldehyde.

Highlights:

- **Economical** – less expensive than KLH
- **Large** – provides an 8 x 10^6 Da scaffold for antigen conjugation
- **Flexible packages** – single use units to gram quantities available
- **Optimized** – streamlined kits which contain all necessary reagents to perform conjugation and clean-up

Ordering Information

Product #	Description	Pkg. Size
77130	**Imject Blue Carrier Protein** Formulation: ~200mg/mL in sterile 0.15M sodium chloride, 0.1M sodium phosphate, pH 7.2	100mg
77660	**Imject Blue Carrier Protein** Formulation: 100 to 200mg/mL in 50% glycerol, 0.15M sodium chloride, 0.1M sodium phosphate, pH 7.2	500mg
77670	**Imject EDC Blue Carrier Protein Spin Kit** *Sufficient for five conjugation reactions* Kit Contents: Imject Blue Carrier Protein (in MES buffer) Imject EDC Conjugation Buffer EDC Crosslinker Imject Purification Buffer Salts Zeba Spin Desalting Columns, 7K MWCO	Kit 5 x 2mg 30mL 5 x 10mg 5 x 0.84g 5 x 2mL

Maleimide Activated Blue Carrier Protein

To target reduced sulfhydryl groups from cysteine residues on your antigen, use maleimide activated Blue Carrier. Derivitazed with the crosslinker Sulfo-SMCC, maleimide activated Blue Carrier is optimized for the highest conjugation efficiency. Targeting cysteine residues on your antigen allow for controlled orientation of the antigen on the carrier protein.

Highlights:

- **Economical** – lower cost alternative to mcKLH
- **Unique** – The only source of pre-activated Blue Carrier
- **Easy to use** – pre-activation means ready to use immediately
- **Targeted** – cysteine reactive chemistry enables controlled orientation of your antigen
- **Flexible** – available in various package sizes
- **Easy to handle** – high solubility to minimize loss during conjugation

Ordering Information

Product #	Description	Pkg. Size
77661	**Imject Maleimide-Activated Blue Carrier Protein** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	2mg
77662	**Imject Maleimide-Activated Blue Carrier Protein** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	10 x 10mg
77664	**Imject Maleimide-Activated Blue Carrier Protein** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	10mg
77665	**Imject Maleimide-Activated Blue Carrier Protein Spin Kit** *Sufficient for five conjugation reactions.* Kit Contents: Imject Maleimide-Activated Blue Carrier Protein Imject Maleimide Conjugation Buffer Imject Purification Buffer Salts Zeba Spin Desalting Columns, 7K MWCO	Kit 5 x 2mg 30mL 5 x 0.84g 5 x 2mL

Imject Bovine Serum Albumin (BSA)

BSA can be used for initial immunizations, but is more commonly used as a non-relevant carrier for ELISA analysis of antibody response. For example, peptide antigens are more easily coated in polystyrene microplates when conjugated to BSA, thereby facilitating the screening of antisera that were produced using immunogen conjugates with KLH or other more immunogenic carrier protein. BSA is considerably smaller than traditional carrier proteins (67kDa), and is derived from mammalian sources.

Highlights:

- **Flexible format** – lyophilized in either PBS or MES buffer
- **Pure** – purified Fraction V BSA,
- **Optimized** – available in all inclusive spin kits
- **Custom** – Available as underivatized or maleimide activated

Ordering Information

Product #	Description	Pkg. Size
77110	**Imject BSA (in PBS)** Formulation: Lyophilized from 10mg/mL protein solution in phosphate-buffered saline with stabilizer, pH 7.2	5 x 20mg
77171	**Imject BSA (in MES Buffer)** Formulation: Lyophilized from 10mg/mL protein solution in 50mM MES, 10mM sorbitol, pH 4.7	2mg
77672	**Imject EDC BSA Spin Kit** *Sufficient for five conjugation reactions.* Kit Contents: Imject BSA (in MES buffer) Imject EDC Conjugation Buffer EDC Crosslinker Imject Purification Buffer Salts Zeba Spin Desalting Columns, 7K MWCO	Kit 5 x 2mg 30mL 5 x 10mg 5 x 0.84g 5 x 2mL
77116	**Imject Maleimide-Activated BSA** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	2mg
77115	**Imject Maleimide-Activated BSA** Formulation: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose, pH 7.2	10mg
77667	**Imject Maleimide-Activated BSA Spin Kit** *Sufficient for five conjugation reactions.* Kit Contents: Imject Maleimide-Activated BSA Imject Maleimide Conjugation Buffer Imject Purification Buffer Salts Zeba Spin Desalting Columns, 7K MWCO	Kit 5 x 2mg 30mL 5 x 0.84g 5 x 2mL

Reference
Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp. 56-100. (Product #15050) *This manual discusses the use of carrier proteins in detail.*

Cationized BSA

Thermo Scientific Imject cBSA can be used with both haptens and full-sized protein antigens to elicit an enhanced immunological response toward a coupled molecule. The use of cBSA as a carrier for both proteins and peptides eliminates the need to develop protocols for each antigen system. Thermo Scientific Imject cBSA will enhance the antibody response to large proteins as well as haptens conjugated to it. Native OVA generates a lower antibody titer than OVA conjugated to cBSA (Figure 13). The cBSA System is so potent that a second booster immunization may not be necessary.

Highlights:

- Unique cationized BSA carrier enhances immune response for both haptens and large proteins
- Stronger immune response than BSA or OVA; no need for Freund's Complete Adjuvant
- Enhanced antibody response of long duration
- Does not aggregate during EDC conjugation
- So potent that a second booster immunization may not be necessary

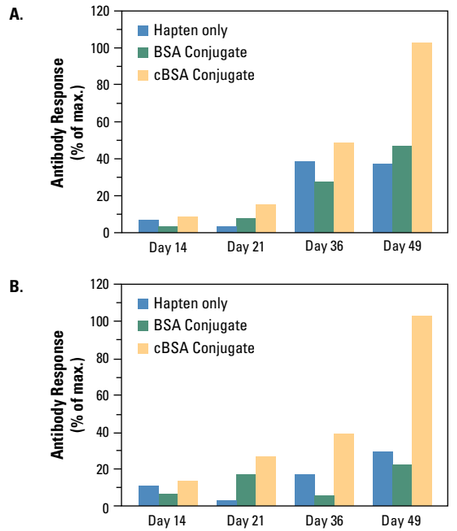


Figure 13. Antibody response to ovalbumin.

Ordering Information

Product #	Description	Pkg. Size
77150	**Imject cBSA (in PBS)**	10mg
77165	**Imject cBSA (in MES buffer)**	2mg
77155	**Imject Maleimide Activated cBSA**	10mg

References
Briggs, S.D., *et al.* (2001). *Genes Dev.* **15,** 3286-3295.
Domen, P.L., *et al.* (1987). *J. Immunol.* **139,** 3195-3198.
Hachiya, A., *et al.* (2002). *J. Biol. Chem.* **277,** 5395-5403.
Muckerheide, A., *et al.* (1987). *J. Immunol.* **138,** 833-837.
Muckerheide, A., *et al.* (1987). *J. Immunol.* **138,** 2800-2804.

Imject Ovalbumin (OVA)

Thermo Scientific Imject OVA is purified ovalbumin protein conveniently formulated for preparation of hapten-carrier conjugates. Because the protein is only weakly immunogenic, its primary use in antibody production workflows is as a secondary (independent) carrier protein to make antigens more amenable to antibody screening procedures. For example, peptide antigens are more easily coated in polystyrene microplates when conjugated to ovalbumin, thereby facilitating the screening of antisera that were produced using immunogen conjugates with KLH or other more immunogenic carrier protein.

Purified, carrier-grade ovalbumin is also available modified with SMCC crosslinker to attach maleimide groups capable of forming covalent crosslinks with sulfhydryl (-SH) moieties on cysteine residues of peptides and other molecules. Immunogens are easily prepared from peptide antigens that have been engineered with a terminal cysteine residue as a precise conjugation point. Several antigen molecules can be attached per carrier protein molecule without blocking intended epitopes by crosslinking to primary amines or carboxylates within the peptide sequence.

Highlights:

- Purified from chicken egg whites; available lyophilized in PBS or MES buffer
- Single polypeptide protein with molecular mass of 45,000 Daltons
- Contains 20 lysine residues, most of which have primary amines that are capable of reacting with a conjugation reagent
- Contains 14 aspartic acid and 33 glutamic acid residues whose carboxyl groups account for the net negative charge (pI 4.63) of OVA
- Not as immunogenic as keyhole limpet hemocyanin (KLH) but more soluble in DMSO (dimethyl sulfoxide) for applications that require this solvent
- Useful as an irrelevant protein carrier for antibody screening and immunoassays after using KLH as the carrier protein to generate the immune response against the hapten

Ordering Information

Product #	Description	Pkg. Size
77120	Imject Ovalbumin (in PBS)	5 x 20mg
77126	Imject Maleimide-Activated Ovalbumin	2mg

Reference

Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp. 56-100. (Product #15050) *This manual discusses the use of carrier proteins in detail.*

Adjuvants and Immunization

Adjuvants

To enhance the immune response to an immunogen, various additives called adjuvants can be used. When mixed and injected with an immunogen, an adjuvant will enhance the immune response. An adjuvant is not a substitute for a carrier protein because it enhances the immune response to immunogens but cannot itself render haptens immunogenic. Adjuvants are nonspecific stimulators of the immune response, helping to deposit or sequester the injected material and causing a dramatic increase in the antibody response.

There are many popular adjuvants, including Freund's complete adjuvant (FCA). This reagent consists of a water-in-oil emulsion and killed Mycobacterium. The oil-and-water emulsion localizes the antigen for an extended period of time, and the Mycobacterium attracts macrophages and other appropriate cells to the injection site. Freund's complete adjuvant is used for the first injection (immunization). Subsequent boosts use immunogen in an emulsion Freund's incomplete adjuvant (FIA), which lacks the Mycobacterium. Freund's adjuvants are very effective, but they do pose risks to both animal and researcher because of the toxic mycobacterial components.

Solutions of aluminum hydroxide (alum) are convenient alternatives to Freund's adjuvants. Alum is considerably easier to mix with immunogens than Freund's adjuvants, as it does not require laborious emulsification. It is not as strong of a stimulator as Freund's complete adjuvant (FCA), as is less likely to elicit an immune response for a completely non-immunogenic compound. However, the vast majority of peptide-carrier protein conjugates are immunogenic, and alum provides significant stimulation for them. Alum is safer to use than Freund's adjuvants, as it is much less likely to cause tissue necrosis at the injection site.

Immunization Protocols

The concentration of the immunogen before mixing with adjuvant will ultimately determine the amount of conjugate that will be administered per injection. The following protocols have been proven successful for injection and bleeding. The schedules can be customized for your convenience or when the condition of the animals warrants such consideration. In any case, injections should be discontinued whenever a severe reaction is observed in the animals–either locally or systemically. Only qualified and certified personnel should perform these animal procedures. Individuals not familiar with these techniques should consult an experienced investigator for training before attempting to immunize and bleed animals.

Immunization Schedule for Mice

- Day 0: Collect pre-immune serum from the mouse to use as a blank when performing ELISA screening after immunization. Store frozen. Inject 50 to 100µg of immunogen (equal to 100 to 200µL of antigen-adjuvant mixture) per mouse. Typical routes of injection include intraperitoneal (i.p.) or subcutaneous (s.c.). One or two such injections may be made per animal.
- Day 14: Boost with an equivalent amount of immunogen in adjuvant.
- Day 21: Test bleed and assay antibody response by ELISA. (Typically, mice are bled under anesthesia through the tail vein or the retro-orbital plexis).
- Day 28: Boost again if necessary. Continue with a similar schedule of alternating boosts and test bleeds until a satisfactory response is observed. For monoclonal antibody production, inject either i.p. or intravenously (i.v.) 4 to 5 days before fusion with the immunogen dissolved in saline (no adjuvant).

Immunization Schedule for Rabbits:

- Day 0: Collect pre-immune serum from the rabbit to use as a blank when performing ELISA after immunization. Store frozen. Inject 100µg of immunogen (equal to about 200µL of the antigen-adjuvant mixture) into each of 8 to 10 subcutaneous sites on the back of the rabbit. Other routes of injection may also be used, but this is by far the easiest with the rabbit.
- Day 14: Boost with an equivalent amount of adjuvant.
- Day 21: Test bleed and assay antibody response by ELISA. (Typically, rabbits are bled through the ear vein without anesthetic). It is not difficult to collect 5 to 10mL of blood, which is more than adequate for measuring antibody response.
- Day 28: Boost again if necessary. Continue with a similar schedule of alternating boosts and test bleeds until a satisfactory response is observed.

General references about antibody production

1. Benjamini, E., *et al.* (1991). *Immunology, A Short Course, Second Ed.* Wiley-Liss, New York, NY.
2. Germain, R. (1986). The ins and outs of antigen processing and presentation. *Nature* **322**: 687-689.
3. Sell, S. (1987). *Immunology, Immunopathology, and Immunity.* Elsevier, New York, NY.
4. Bartel, A. and Campbell, D. (1959). Some immunochemical differences between associated and dissociate hemocyanin. *Arch. Biochem. Biophys.* **82**: 2332.
5. Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory. (Part No. 15050). **Chapter 5** discusses the use of carrier proteins.
6. Hermanson, G.T. (2008). *Bioconjugate Techniques.* 2nd edition, Academic Press, New York. (Part No. #20036). **Chapter 19** discusses carrier protein uses and immunogen preparation.
7. Oliva H., *et al.* (2002) Monoclonal antibodies to molluskan hemocyanin from *Concholepas concholepas* demonstrate common and specific epitopes among subunits. *Hybridoma and Hybridomics.* **21**: 365-373.
8. Muckerheide, A., *et al.* (1987). Cationization of protein antigens. I. Alteration of immunogenic properties. *J. Immunol.* **138**: 833-837.
9. Muckerheide, A., *et al.* (1987). Cationization of protein antigens. II. Alteration of regulatory properties. *J. Immunol.* **138**: 2800-2804.
10. Domen, P.L., *et al.* (1987). Cationization of protein antigens. III. Abrogation of oral tolerance. *J. Immunol.* **139**: 3195-3198.
11. Apple, R.J., *et al.* (1988). Cationization of protein antigens. IV. Increased antigen uptake by antigen-presenting cells. *J. Immunol.* **140**: 3290-3295.

Ordering Information

Product #	Description	Highlights	Disadvantages	Reference(s)	Pkg. Size
77140	Imject Freund's Complete Adjuvant (FCA)	• Enhances immune response to immunogen • Used for initial injections • Water-in-oil emulsion and killed Mycobacterium	• Difficult to mix with immunogen • Can cause tissue necrosis at injection site	1-3	5 x 10mL
77145	Imject Freund's Incomplete Adjuvant (FIA)	• Used for subsequent boosts after initial injection • No Mycobacterium present; mix with immunogen	• Difficult to mix with immunogen	3	5 x 10mL
77161	Imject Alum	• Unsurpassed convenience – ready for injection • Specially made suspension of aluminum hydroxide and magnesium hydroxide allows you to mix your antigen with Imject Alum • Ideal for use with Imject cBSA	• Does not elicit as strong an immune response as FCA	6-8	50mL

Determining Antibody Concentration

Concentration vs. Titer

Successful and reproducible antibody labeling and immunoassays are contingent on accurate information about the concentration and functional titer of purified antibodies. Concentration and titer are not equivalent. Concentration is the total amount of antibody without regard to its function. Depending upon the methods of purification employed, only a percentage of the total antibody concentration is composed of intact, active and functioning antibody with regard to its ability to bind antigen and yield a measurable response in an immunoassay. The titer of an antibody is the useful dilution of antibody in a given immunoassay. This is determined as the greatest dilution of an antibody preparation that yields a response in that assay through a series of dilutions and is a functional measure of the activity of that antibody preparation. Some knowledge of both the concentration and titer is often helpful in optimizing the purification of an antibody and in subsequent use.

Antibody Concentration

The concentration of pure antibodies can be estimated from the absorbance measured at 280nm, using an extinction coefficient of 13.5 for a 1% solution of IgG (10mg IgG/mL). The concentration of pure antibodies can also be measured using a protein assay such as the Thermo Scientific BCA Protein Assay (Product # 23225) or Coomassie Plus Assay Kit (Product # 23236). Using an immunoglobulin of known concentration (e.g., bovine gamma globulin, Product # 23212) as a standard, accurate determination of antibody concentration is possible with these protein assays.

Often, antibodies are not available in purified form and must be quantitated in serum, ascites fluid or culture supernatants. The increased use of antibodies as tools for research, diagnostic and therapeutic purposes has led to a demand for methods that can accurately determine antibody concentrations in these heterogeneous mixtures.

The Thermo Scientific Easy-Titer Assay Kits are simple, mix-and-read assays that allow the accurate determination of antibody concentrations from 15-300ng/mL in about 30 minutes. The assay uses monodispersed polystyrene beads that bind to specific antibodies and absorb light at 340 and 405nm. When the beads are mixed with a sample containing their target antibody, they aggregate and their ability to absorb light is decreased (Figure 14). Because this is an aggregation assay, low antibody concentrations yield high absorbance values, while high antibody concentrations yield low absorbance values. The decrease is proportional to antibody concentration and a standard curve can be generated to accurately quantitate levels of IgG in a variety of samples. Our Easy-Titer® Assay Kits feature a simple procedure that reduces hands-on time by using fewer steps and leads to more reproducible results. The antibody-binding beads are added to each well of a 96-well plate. Sample containing antibody is added to the wells, and this mixture is incubated for 5 minutes. Blocking reagent is added to the wells and the plate is incubated for another 5 minutes. The absorbance of each well at 340 or 405nm is read on a 96-well plate reader. The entire process can be completed easily in about 30 minutes, unlike standard ELISA techniques that can require several hours.

Each Easy-Titer Assay Kit is specific for a particular antibody species and isotype. For example, the Easy-Titer Human IgG Assay Kit is specific for the human gamma chain and yields a uniform response with human IgG molecules of all subclasses (IgG_1, IgG_2, IgG_3 and IgG_4). It does not cross-react with other classes of human antibodies (IgM, IgA, IgD and IgE). In addition, the kit does not cross-react with antibodies from other species such as bovine antibodies present in the media used to culture antibody-producing hybridoma cells. This remarkable specificity allows the measurement of human IgG concentrations from a variety of sample types such as culture supernatants, ascites or body fluids without first purifying the antibody from other contaminants.

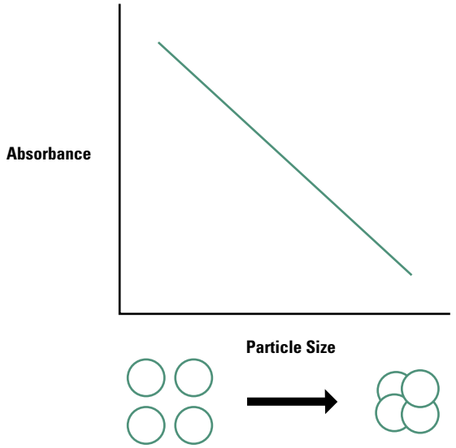
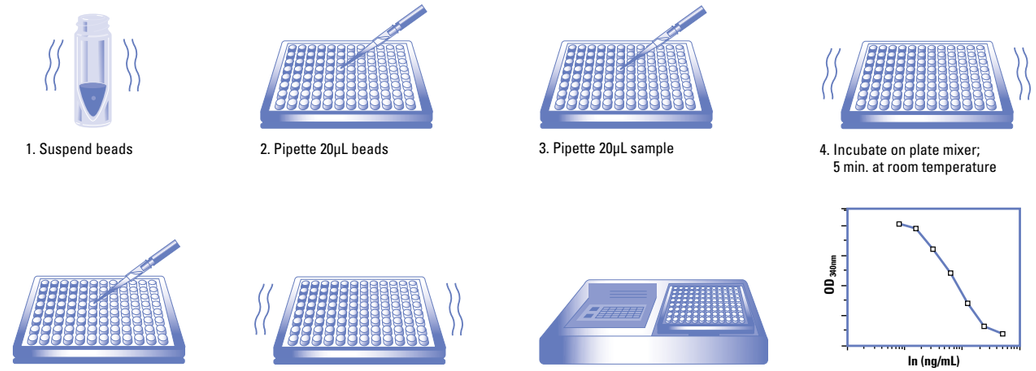


Figure 14. The assay principle behind the Thermo Scientific Easy-Titer Human IgG Assay Kit.



Protocol for the Thermo Scientific Easy-Titer Human IgM Assay Kit.

Easy-Titer IgG Assay Kits

The fastest, easiest way to quantitate antibodies … ever!

It is no longer necessary to wait on or to rely on inaccurate and insensitive UV or colorimetric IgG determination methods. It is even possible to avoid the tedious time-consuming ELISA approach to determine antibody concentration. Thermo Scientific Easy-Titer IgG Assay Kits make it possible to detect IgG in less time and with greater specificity and sensitivity that ever before.

Highlights:

- Fast – 10-minute total assay time
- Detection requires only measuring absorbance at 340 or 405nm
- Sufficient for 96 assays – up to 87 determinations and a standard curve on a single plate
- Ready-to-use, three-component kit is easy to use
- Requires only a microplate, a shaker and a microplate reader
- Measures antibodies from culture supernatants, ascites or body fluids
- Measures humanized antibodies and chimeras with intact Fc regions

How the assay works:

- Monodisperse beads sensitized with a specific antibody absorb at 340 and 405nm
- The beads agglutinate in the presence of human IgG or IgM
- Larger diameter clusters form that absorb less efficiently at 340 and 405nm
- This decrease in absorbance is proportional to antibody concentration

Human IgG Assays

- Specificity
  - Specific for human IgG (all subclasses)
  - No cross-reactivity with human IgA, IgD, IgE or IgM or with IgG from other species
- Sensitivity
  - 15ng/mL detection limit
  - 15-300ng/mL detection range
- Coefficient of variation
  - <5% intra- and interassay

Human IgM Assay

- Specificity
  - Specific for human IgM
  - No cross-reactivity with human IgG or with IgM from other species
- Sensitivity
  - 15ng/mL detection limit
  - 15-300ng/mL detection range
- Coefficient of variation
  - <5% intra- and interassay

Our Easy-Titer Assay Kits do not cross-react with antibodies from other species such as bovine antibodies present in the media used to culture antibody-producing hybridoma cells. This remarkable specificity allows the measurement of human IgG concentration from a variety of sample types such as culture supernatants, ascites or body fluids without first purifying the antibody from other contaminants.

Ordering Information

Product #	Description	Pkg. Size
23310	**Easy-Titer Human IgG (H+L) Assay Kit** *Sufficient material for 96 individual microplate assays, (8 assays and 1 standard curve)* Includes: Goat Anti-Human IgG Sensitized Polystyrene Beads [Monodisperse, polystyrene IgG (Fc) sensitized beads are supplied suspended in a phosphate buffer, pH 7.4 and stabilized with BSA and 0.1% azide] Easy-Titer Dilution Buffer Easy-Titer Blocking Buffer **Human IgG Standard and microplates are available separately (see below).**	Kit 2mL 30mL 15mL
23325	**Easy-Titer Human IgG (gamma chain) Assay Kit** Includes: Goat Anti-Human IgG (γ chain) Sensitized Beads Easy-Titer Dilution Buffer Easy-Titer Blocking Buffer	Kit 2mL 30mL 15mL
23315	**Easy-Titer Human IgM Assay Kit** Includes: Goat Anti-Human IgM Sensitized Polystyrene Beads Easy-Titer Dilution Easy-Titer Blocking Buffer	Kit 2mL 30mL 15mL

We also offer the same easy-to-use assay technology for Mouse and Rabbit IgG

23300	**Easy-Titer Mouse IgG Assay Kit** Key Component: Goat Anti-Mouse IgG Sensitized Beads	Kit 2mL
23305	**Easy-Titer Rabbit IgG Assay** Key Component: Goat Anti-Rabbit IgG Sensitized Beads	Kit 2mL

IgG Standards for Easy-Titer Kits

31154	**Human IgG, Whole Molecule**	10mg
31146	**Human IgM, Whole Molecule**	2mg
31202	**Mouse IgG, Whole Molecule**	10mg
31235	**Rabbit IgG, Whole Molecule**	10mg

Microplates

15041	**Pierce 96-Well Plates – Corner Notch**	100 plates
15031	**Pierce 8-Well Strip Plates – Corner Notch**	100 plates

Isotyping Antibodies

Importance of Isotype Determination

Determining the class and subclass identity of an antibody is especially important for choosing by what method it should be purified and used in immunoassays. For example, if an antibody is determined to be IgM, it cannot be purified effectively with Protein A or G, and it will most likely require fragmentation for use in immunohistochemical procedures. If a monoclonal antibody is determined to be IgG_1 composed of kappa light chains, there is a good possibility that immobilized Protein L can be used to purify it from culture supernatant without contamination of bovine immunoglobulins from the serum supplement.

Rapid Isotyping Kit

Quickly and easily determine mouse monoclonal antibody (MAb) class and subclass identity.

The Thermo Scientific Pierce Rapid Isotyping Kits are quick and accurate lateral-flow assays for monoclonal antibody class and subclass determination. The kits are composed of small cartridges that provide a color-readout of the monoclonal antibody isotype within five minutes after pipetting a small amount of diluted culture supernatant or ascites fluid sample into the sample well. The kits determine mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgA, IgM and kappa and lambda light chains.

The assay is performed by simply adding a properly diluted tissue culture supernatant or mouse ascites sample to the well of the small cassette. Gold conjugates embedded in the cassette form specific class- and subclass-soluble complexes with the antibodies in the sample. These complexes travel the length of the membrane and are resolved on the anti-isotype and class-specific antibody-impregnated membrane. Results are displayed as a red band indicating the antibody isotype or subclass.

Determining the class and subclass of a monoclonal antibody is useful in planning the best immunoglobulin purification method. For example, mouse IgA and IgM are best purified by size (i.e., gel exclusion) or using immunoaffinity separation columns. Mouse IgG_{2a} and IgG_{2b} are purified with immobilized Protein A at pH 7-8, while Mouse IgG_1 binds best to Protein A at pH 8-9. Immunoglobulin that contains kappa light chains can be purified using immobilized Protein L.

Determining the class and subclass of a monoclonal antibody is useful in planning the best immunoglobulin purification method. For example, mouse IgA and IgM are best purified by size (i.e., gel exclusion) or using immunoaffinity separation columns. Mouse IgG_{2a} and IgG_{2b} are purified with immobilized Protein A at pH 7-8, while Mouse IgG_1 binds best to Protein A at pH 8-9. Immunoglobulin that contains kappa light chains can be purified using immobilized Protein L.

Highlights:

- **Long shelf life** – Stable for > 1 year at room temperature
- **Single-step** – Add diluted antibody sample to the loading-well of cassette
- **Fast** – Within 5 minutes a band appears that indicates the antibody isotype
- **Sensitive** – Detect antibodies in tissue culture media or ascites fluid samples at any concentration greater than 10ng/mL
- **Specific** – Assay does not cross-react with fetal bovine serum (FBS)
- **Reliable** – Results are similar to the standard ELISA-based isotyping assays

Pierce Rapid Isotyping Kits are compatible with both tissue culture supernatant and mouse ascites fluid. They are more sensitive than conventional latex-bead based dipstick assays and much faster than ELISA-based isotyping assays. Kits are available for isotyping mouse Ig_{G1}, IgG_{2a}, IgG_{2b}, IgG_3, IgA, IgM and kappa and lambda light chains and require only 0.5µL ascites fluid, 5-55µL of cell culture supernatant or 1.5ng of purified antibody.

Ordering Information

Product #	Description	Pkg. Size
26178	**Pierce Rapid Antibody Isotyping Kit – Mouse** *Sufficient reagents for 10 samples containing mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgA, IgM.* Kit contains: Isotyping Cassette (two per pouch) Sample Diluent	Kit 10 pouches 45mL
26179	**Pierce Rapid Antibody Isotyping Kit plus Kappa and Lambda – Mouse** *Sufficient reagents for 10 samples containing mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgA, IgM and kappa and lambda chains.* Kit contains: Isotyping Cassette (three per pouch), Sample Diluent	Kit 10 pouches 45mL

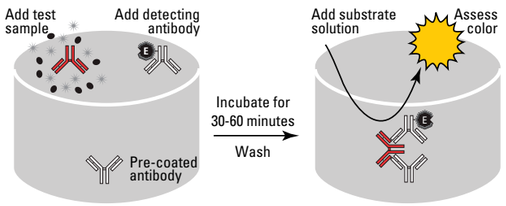
Rapid ELISA Mouse mAb Isotyping Kit

Trouble-free determination of mouse antibody class and subclass identity for single or multiple samples.

The Thermo Scientific Pierce Rapid ELISA Mouse mAb Isotyping Kit enables easy identification of mouse immunoglobulin class, subclass and light chain. This fast assay uses ELISA strip-plates pre-coated in different wells with anti-mouse heavy-chain capture antibodies (anti-IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgA and IgM) or anti-mouse light-chain capture antibody (kappa or lambda). This approach eliminates the need to purify and immobilize an antigen to determine immunoglobulin isotype. A mouse monoclonal antibody sample applied to the wells can be isotyped within one hour. Results are evaluated qualitatively by visual inspection or quantitatively by measuring the absorbance at 450nm.

Highlights:

- **Fast** – determine antibody isotype in ~1 hour; considerably faster than traditional ELISA kits
- **Convenient** – eight-well strip format allows partial use of the plate; use one strip (column) for each sample (12 samples per plate)
- **Specific** – characterize antibodies for six different subclasses and two different light-chain types
- **Sensitive** – accurate characterization with samples containing >3ng/mL of test antibody
- **Flexible** – kit is compatible with hybridoma cell culture supernatant, ascites fluid or purified antibodies
- **Cost effective** – cost per assay is less than with single-use assay strips or cassettes
- **No special equipment needed** – assess results visually or measure quantitatively using an ordinary ELISA plate reader (450nm)
- **Complete and easy to use** – includes precoated plates, detecting antibody, buffers, and TMB substrate and stop solutions



Procedure for the Thermo Scientific Pierce Rapid ELISA Isotyping Kit.

The simple ELISA procedure involves only one probing-incubation step. Sample and detection antibody are added together to the wells of the precoated microplate. (Each well in an eight-well strip is coated with a different type-specific capture antibody.) After 30-60 minutes, TMB substrate is added to reveal the antibody isotype based on which wells in the strip produce color.

Ordering Information

Product #	Description	Pkg. Size
37503	**Pierce Rapid ELISA Mouse mAb Isotyping Kit** *Five microplates and sufficient reagents to isotype 60 mouse monoclonal antibodies.*	Kit
	Includes: Pre-coated 96-well Isotyping Plates	5 plates
	Goat Anti-Mouse IgG+IgA+IgM-HRP Conjugate	30mL
	TMB Substrate	55mL
	Stop Solution	55mL
	30X Wash Buffer	200mL
	Tris buffered Saline	2 packs

Immunodiffusion Plates

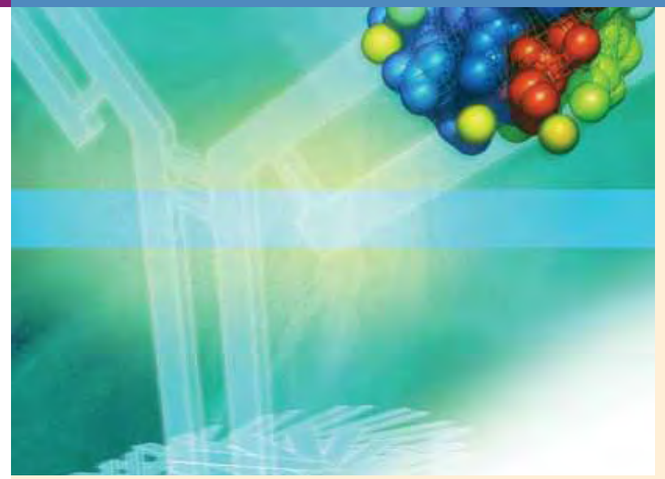
Pre-cast Thermo Scientific Immunodiffusion Plates provide antibody-antigen precipitation detection.

Highlights:

- Gelling Agent contains precipitin brighteners, along with diffusion enhancers that help speed the interaction process
- Good precipitin bands from all species (including rabbit)
- Gels can be washed, dried and stained for a permanent record

Ordering Information

Product #	Description
31111	**Immunodiffusion Plates, Agarose Gelling Agent** 4 pattern/plate, 6 plates/pkg.
31113	**Immunodiffusion Plates, Agarose Gelling Agent** 1 pattern/plate, 10 plates/pkg.



Antibody Purification Overview

Antibodies specific for an antigen of interest are one of the most useful and important tools that biology researchers can possess. The production and use of specific antibodies as detection probes and purification ligands (i.e., immunotechnology) has revolutionized bioresearch and diagnostic technologies. Animals immunized with prepared antigens will produce specific antibodies against the antigen. When purified from serum or hybridoma cell lines that are prepared from tissue of the immunized animal, the antibody can be used directly (or after labeling with enzyme or fluorescent tags) to probe the specific antigen in Western blotting, ELISA or a variety of other applications. Antibodies are most commonly purified by one of two affinity purification methods: general immunoglobulin purification (pages 28-45) or specific antibody purification (see pages 46-50). See also Table 1.

General Purification of Immunoglobulins

Because antibodies have predictable structure, including relatively invariant domains, it has been possible to identify certain protein ligands that are capable of binding generally to antibodies, regardless of the antibody's specificity to antigen. Protein A, Protein G and Protein L are three bacterial proteins whose antibody-binding properties have been well characterized. These proteins have been produced recombinantly and used routinely for affinity purification of key antibody types from a variety of species. A genetically engineered recombinant form of Protein A and G, called Protein A/G, is also available. These antibody-binding proteins are available immobilized to beaded agarose resin, Thermo Scientific UltraLink Biosupport and coated onto microplates.

Proteins A, G, A/G and L bind to antibodies at sites other than the antigen-binding domain. Therefore, these proteins can be used in purification schemes such as immunoprecipitation.

Proteins A, G, A/G and L have unique properties, which make each one suitable for different types of antibody targets (e.g., antibody subclass or animal species). It is important to realize that use of Protein A, G or L results in purification of general immunoglobulin from a crude sample. Depending on the sample source, antigen-specific antibody may account for only a small portion of the total immunoglobulin in the sample. For example, generally only 2-5% of total IgG in mouse serum is specific for the antigen used to immunize the animal.

Table 1. Antibody Purification Methods.

	Purification Type	Description	Available Thermo Scientific Support
General	Negative selection	Removal of all non-immunoglobulin proteins from a serum sample	Melon Gel
	IgG enrichment	Immobilized globulin binding proteins to selectively remove IgG from a serum sample	Immobilized Protein A
			Immobilized Protein G
			Immobilized Protein A/G
			Immobilized Protein L
	IgG enrichment	Thiophilic adsorption	Thiophilic Adsorbent
	IgM enrichment	Use Mannan binding protein to selectively isolate IgM	Immobilized Mannan Binding Protein
	IgA enrichment	Use Jacalin, a D-galactose binding lectin, to selectively isolate IgA	Immobilized Jacalin
	IgY enrichment	Delipidation and precipitation from egg yolks	IgY Precipitation Reagent
Specific	Affinity purification	Create an affinity column with the antigen used to immunize the animal.	NHS-ester Activated Agarose - for immobilizing antigens via primary amines
			AminoLink Plus Agarose - for immobilizing antigens via primary amines
			SulfoLink Resin - for immobilizing antigens via reduced sulfhydryls
			CarboxyLink Resin - for immobilizing antigens via carboxylic acids

Immobilized Protein L, Protein A, Protein G and Protein A/G

We offer these popular antibody-binding proteins immobilized on several different resins, beads and plates for use in immunoaffinity purification techniques. All four proteins (A, G, A/G and L) are available as purified recombinants immobilized to crosslinked 6% beaded agarose. This is the traditional format historically used for small-scale column purification and immunoprecipitation methods. Our agarose resins differ from those typically offered by other suppliers in that our immobilization method is more stable and results in less nonspecific binding. We also offer "Plus" versions of the Protein A, G, A/G and L agarose resins, which contain twice the amount of protein per milliliter of resin and provide for nearly twice the antibody binding capacity.

Protein A, G, A/G and L are also available immobilized to UltraLink® Biosupport, an extremely durable, polyacrylamide-based resin with very low nonspecific binding characteristics. The UltraLink Format is a perfect support for working with large volume samples in large-scale purification methods requiring fast flow and high pressure.

The interaction between the various proteins and IgG is not equivalent for all species or all antibody subclasses. The tables on the following page will help you decide which affinity protein is best for your application (Tables 2 and 3).

Table 2. Characteristics of immunoglobulin-binding proteins.

	Recombinant Protein L	Native Protein A	Recombinant Protein A	Recombinant Protein G	Recombinant Protein A/G
Production Source	*E. coli*	*S. aureus*	*E. coli*	*E. coli*	*E. coli*
Molecular Weight	35,800	46,700	44,600	21,600	50,460
Number of Binding Sites for IgG	4	4	4	2	6
Albumin-Binding Site	No	No	No	No	No
Optimal Binding pH	7.5	8.2	8.2	5	5-8.2
Binds to	V_LK	F_c	F_c	F_c	F_c

Table 3. Binding characteristics of immunoglobulin-binding proteins and Thermo Scientific Thiophilic Adsorbent.*

	Protein A	Protein G	Protein A/G	Protein L†	Thiophilic Adsorbent
Human IgG	s	s	s	s	m
Mouse IgG	s	s	s	s	s
Rabbit IgG	s	s	s	w	m
Goat IgG	w	s	s	nb	s
Rat IgG	w	m	m	s	s
Sheep IgG	w	s	s	nb	s
Cow IgG	w	s	s	nb	s
Guinea Pig IgG	s	w	s	–	s
Hamster IgG	m	m	m	s	–
Pig IgG	s	w	s	s	s
Horse IgG	w	s	s	–	s
Donkey IgG	m	s	s	–	–
Dog IgG	s	w	s	–	s
Cat IgG	s	w	s	–	s
Monkey IgG (Rhesus)	s	s	s	–	s
Chicken IgY	nb	nb	nb	nb	m
Human IgM	w	nb	w	s	m
Human IgE	m	nb	m	s	–
Human IgD	nb	nb	nb	s	–
Human IgA	w	nb	w	s	m
Human IgA_1	w	nb	w	s	m
Human IgA_2	w	nb	w	s	m
Human IgG_1	s	s	s	s	m
Human IgG_2	s	s	s	s	m
Human IgG_3	w	s	s	s	m
Human IgG_4	s	s	s	s	m
Human Fab	w	w	w	s	m
Human ScFv	w	nb	w	s	m
Mouse IgG_1	w	m	m	s	s
Mouse IgG_{2a}	s	s	s	s	s
Mouse IgG_{2b}	s	s	s	s	s
Mouse IgG_3	s	s	s	s	s
Rat IgG_1	w	m	m	s	s
Rat IgG_{2a}	nb	s	s	s	s
Rat IgG_{2b}	nb	w	w	s	s
Rat IgG_{2c}	s	s	s	s	s
Cow IgG_1	w	s	s	nb	s
Cow IgG_2	s	s	s	nb	s
Sheep IgG_1	w	s	s	nb	s
Sheep IgG_2	s	s	s	nb	s
Goat IgG_1	w	s	s	nb	s
Goat IgG_2	s	s	s	nb	s
Horse IgG(ab)	w	nb	w	–	s
Horse IgG(c)	w	nb	w	–	s
Horse IgG(T)	nb	s	s	–	s
Mouse IgM	nb	nb	nb	s	m

w = weak binding, m = medium binding, s = strong binding, nb = no binding, – means information not available

* *Data represent a summary of binding properties reported in the literature. Inevitably some discrepancies exist among reported values as a result of differences in binding buffer conditions and form of the proteins used.*

† *Binding will occur only if the appropriate kappa light chains are present. Antibodies lambda light chains will not bind, regardless of their class and subclass.*

Protein A

Protein A Beads – Quick Reference

Protein A	Native protein purified from *Staphylococcus aureus* (46.7kDa; four IgG-binding sites)
Specificity (Table 3)	Best for polyclonal IgG from rabbit, pig, dog, cat serum; poor for Mouse IgG1, human IgG3, rat, goat, cow
Supports Offered	Crosslinked 6% beaded agarose UltraLink Biosupport Trisacryl GF-2000 Resin Magnetic particles (1-4µm)
Package Formats	Resin slurries (3 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) NAb IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze

Protein A Characteristics and IgG Binding Properties

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus*. It consists of a single polypeptide chain (MW 46.7kDa) and contains little or no carbohydrate.[1] Protein A binds specifically to the Fc region of immunoglobulin molecules, especially IgG. It has four high-affinity ($K_a = 10^8\ M^{-1}$) binding sites that are capable of interacting with the Fc region of IgGs of several species.[2] The molecule is heat-stable and retains its native conformation even after exposure to denaturing reagents such as 4 M urea, 4 M thiocyanate and 6 M guanidine hydrochloride.[3]

In its immobilized form (e.g., covalently coupled to beaded agarose resin), Protein A has been used extensively for isolation of a wide variety of immunoglobulins from several species of mammals. However, the interaction between Protein A and IgG is not equivalent for all animal sources and subclasses of IgG. For example, human IgG_1, IgG_2 and IgG_4 bind strongly to Protein A, while IgG_3 does not bind.[2] In mice, IgG_{2a} , IgG_{2b} and IgG_3 bind strongly to Protein A, but IgG_1 (the dominant subclass in serum) binds only weakly using standard buffer conditions. Most rat IgG subclasses bind weakly or not at all to Protein A. Despite this variability, Protein A is very effective for routine affinity purification of IgG from the serum of many species. It is especially suited for purification of polyclonal antibodies from rabbits.

Weak binding of Protein A to mouse IgG_1 using traditional Tris•HCl or sodium phosphate buffer systems is of particular concern and is one reason to choose Protein G when purifying mouse antibodies. However, we have developed a binding buffer that allows Protein A to bind mouse IgG_1 nearly as well as other subclasses (see subsequent discussion of IgG Binding and Elution Buffers, page 37).

The variable binding properties of Protein A for different subclasses of IgG can be used advantageously to separate one IgG type from another. Antibodies that do not bind to immobilized Protein A can be recovered by collecting the non-bound ("flow-through") fractions during binding and wash steps in an affinity purification procedure. In this way, human IgG_3 and other immunoglobulin subclasses can be isolated from those that do bind to Protein A; however, other IgGs and serum proteins, such as albumin, will also be present in the non-bound fraction. Certain IgM, IgD and IgA molecules also do not bind to Protein A and can be separated from Protein A-binding proteins in the same manner.

Immobilized Protein A Products

Thermo Scientific Pierce Immobilized Protein A is offered on several different solid supports and made available in different binding capacity formats, package sizes and kit formats. Protein A Agarose generally denotes products composed of highly purified Protein A that is covalently coupled to crosslinked 6% beaded agarose resin.

Our Protein A Agarose is available with different densities of Protein A ligand bound to the resin. The standard version has a binding capacity of 12-19mg of human IgG per mL of resin, while the plus version has a binding capacity of > 35mg of human IgG per mL of resin. Both resins exhibit excellent elution properties when used with Pierce Buffer Systems (Figure 1), which generally enable the resin to be regenerated and used for at least 10 rounds of purification. Supplied as a 50% resin slurry in storage buffer, Immobilized Protein A Agarose is the usual choice either for small-scale batch method purification procedures or for packing gravity-flow columns.

Our Immobilized Protein A is also available on Trisacryl® GF-2000, rather than agarose resin. This stable affinity support can withstand the high-throughput volumes required in large-scale purification procedures. In addition, because Trisacryl GF-2000 is a hydrophilic matrix, nonspecific binding of proteins is minimized.

Thermo Scientific Pierce Protein A UltraLink Resin is another alternative for large-scale, high-throughput applications. UltraLink Biosupport is composed of a hydrophilic, crosslinked bis-acrylamide/azlactone copolymer. It has an average bead diameter of 60µm, can withstand pressures exceeding 100 psi and retains good chromatographic properties using flow rates up to 3,000cm/hour. Our Protein A UltraLink Resin is the ideal choice for medium pressure liquid chromatographic systems.

Thermo Scientific Pierce Recombinant Protein A Agarose (Product #s 20365 and 20366) uses a genetically engineered form of Protein A that is produced recombinantly in a nonpathogenic form of *Bacillus*. Non-essential regions have been removed, and five IgG-binding sites are included, resulting in a mass of 44.6kDa. Some researchers believe that the recombinant form should be used if the antibody preparation has strict requirements for being enterotoxin-free. Otherwise, the native form serves as a highly efficient means for purifying antibodies. Our Recombinant Protein A Agarose is also compatible with Pierce Binding and Elution Buffers.

Thermo Scientific NAb Protein A Spin Columns are available in three package/column sizes: 10 x 0.2mL Protein A resin in a 1mL spin column, 5 x 1mL resin in a 5mL spin column, and 1 x 5mL resin in a 22mL spin column. For the greatest convenience, choose NAb™ Protein A Plus Spin Kits (Product #s 89948 and 89978), which include pre-packed columns of our Protein A Plus Agarose, as well as binding, elution and neutralization buffers. These kits contain everything needed to isolate IgG from serum, ascites or cell culture supernatants through a fast and simple process that requires approximately 30 minutes to complete. The columns in the NAb Kits can each be regenerated a minimum of 10 times without a significant loss of binding capacity. NAb Protein A Spin Kits are available in two sizes: 10 x 0.2mL spin column kit and 2 x 1mL spin column kit. The spin format of these columns and kits greatly

reduces the time required to process serum, ascites and cell culture supernatants and produce a purified antibody preparation. The NAb Columns and Kits are also compatible with gravity-flow and vacuum-based purification methods.

The Thermo Scientific Pierce Protein A Chromatography Cartridges (Product #s 89924 and 89925) are designed for fast, consistent separations using a syringe, pump or chromatography system. They are available in 1mL and 5mL sizes. The column inlet and outlet are molded with 1/16" threads, and adaptors are included for coupling directly to most chromatography systems. Luer-Lok® Adaptors are also provided with the columns for simple attachment to a syringe.

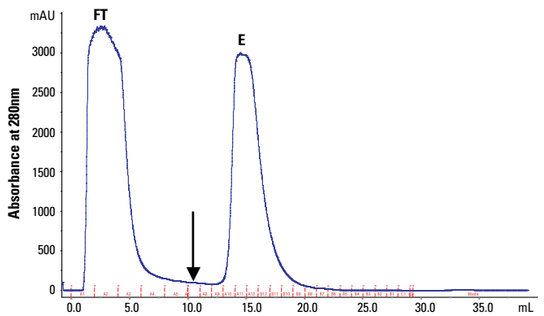


Figure 1. Affinity chromatographic purification of mouse IgG from mouse ascites fluid using Thermo Scientific Pierce Protein A Agarose and the IgG Binding and Elution Buffer System. From 1mL of mouse ascites fluid, 5.5mg of mouse IgG was recovered.

Thermo Scientific MagnaBind Protein A (Product # 21348) is available to perform benchtop magnetic separations quickly and easily. MagnaBind™ Beads consist of a silanized surface over a core of superparamagnetic iron oxide. Protein A has been attached to these beads to allow IgG removal, IgG purification or magnetic immunoprecipitation.

References
1. Sjoquist, J., *et al.* (1972). *Eur. J. Biochem.* **29,** 572-578.
2. Hjelm, H., *et al.* (1975). *Eur. J. Biochem.* **57,** 395-403.
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Immobilized Protein A Products

Ordering Information

Product #	Description	Pkg. Size
20333	**Protein A Agarose** Support: Crosslinked 6% beaded agarose Capacity: 12-19mg human IgG/mL resin	5mL
20334	**Protein A Agarose** Support and Capacity: Same as above	25mL
20356	**Protein A Columns** Support and Capacity: Same as above	5 x 1mL
44667	**Protein A IgG Purification Kit** Includes: Protein A Columns Protein A IgG Binding Buffer IgG Elution Buffer Desalting Columns	Kit 5 x 1mL 1,000mL 500mL 5 x 5mL
20338	**Protein A Trisacryl Resin** Support: Trisacryl GF 2000 Capacity: > 15mg human IgG/mL resin	5mL
53139	**Protein A UltraLink Resin** Support: UltraLink Biosupport Capacity: > 16mg of human IgG/mL resin	5mL

Immobilized Protein A Plus Products

Twice the amount of coupled Protein A per milliliter of resin.

Ordering Information

Product #	Description	Pkg. Size
22810	**Protein A Plus Agarose** Support: Crosslinked 6% beaded agarose Capacity: > 35mg human IgG/mL resin; 16-17mg mouse IgG/mL resin	1mL
22811	**Protein A Plus Agarose** Support and Capacity: Same as above	5mL
22812	**Protein A Plus Agarose** Support and Capacity: Same as above	25mL
89924	**Chromatography Cartridges, Protein A** Support and Capacity: Same as above	2 x 1mL
89925	**Chromatography Cartridge, Protein A** Support and Capacity: Same as above	1 x 5mL
89952	**NAb Protein A Plus Spin Columns** Support and Capacity: Same as above	10 x 0.2mL
89956	**NAb Protein A Plus Spin Columns** Support and Capacity: Same as above	5 x 1mL
89960	**NAb Protein A Plus Spin Column** Support and Capacity: Same as above	1 x 5mL
89948	**NAb Protein A Plus Spin Purification Kit** Includes: Protein A Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer Collection Tubes	Kit 10 x 0.2mL 500mL 50mL 7mL 8 x 2mL
89978	**NAb Protein A Plus Spin Purification Kit** Includes: Protein A Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer	Kit 2 x 1mL 500mL 240mL 7mL
53142	**Protein A UltraLink Plus Resin** Support: UltraLink Biosupport Capacity: > 30mg human IgG/mL resin	5mL
45202	**Protein A Spin Plate** 96–well filter plate containing Protein A Agarose for IgG screening	1 plate

MagnaBind Protein A Beads

Ordering Information

Product #	Description	Pkg. Size
21348	**MagnaBind Protein A Beads** Support: 1-4µm iron oxide particles Capacity: > 0.2mg rabbit IgG/mL beads	5mL

Protein A Coated Microplates

Ordering Information

Product #	Description	Pkg. Size
15130	**Protein A, Clear 96-Well Plates** Coating Volume: 100µL Blocking: SuperBlock Blocking Buffer, 200µL Capacity: ~ 4pmol rabbit IgG/well	5 plates
15132	**Protein A, Clear 8-Well Strip Plates** Specifications: Same as above	5 plates
15154	**Protein A, White 96-Well Plates** Specifications: Same as above	5 plates
15155	**Protein A, Black 96-Well Plates** Specifications: Same as above	5 plates

References
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Protein A Agarose

Our immobilized Protein A, manufactured with a leak-resistant linkage.

Ordering Information

Product #	Description	Pkg. Size
20365	**Recombinant Protein A Agarose** Support: Crosslinked 6% beaded agarose resin Capacity: ≥ 12mg human IgG/mL resin using the IgG Buffer System	5mL
20366	**Recombinant Protein A Agarose** Support and Capacity: Same as above	25mL
22810	**Pierce Protein A Plus Agarose** Support: Crosslinked 6% beaded agarose Capacity: > 34mg human IgG/mL of resin; 16-17mg mouse IgG/mL of resin	1mL

Protein G

Protein G Beads – Quick Reference	
Protein G	Recombinant protein expressed in *E. coli* (21.6kDa; two IgG binding sites)
Specificity (Table 3)	Best for IgG from mouse, human, cow, goat and sheep; poor for guinea pig, pig, dog and cat
Supports Offered	Crosslinked 6% beaded agarose UltraLink Biosupport Magnetic particles (1-4µm)
Package Formats	Resin slurries (3 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) Nab IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze

Protein G Characteristics and IgG Binding Properties

Protein G is a bacterial cell wall protein isolated from group G streptococci.[1] Like Protein A from *Staphylococcus aureus*, Protein G binds to most mammalian immunoglobulins primarily through their Fc regions. Protein G binds weakly to Fab fragments.[1] Sequencing of DNA that encodes native Protein G indicates that there are two immunoglobulin binding sites, as well as albumin and cell surface binding sites.[2] In the recombinant form of Protein G, these albumin and cell surface binding sites have been eliminated to reduce nonspecific binding when purifying immunoglobulins. With the albumin site removed, recombinant Protein G can be used to separate albumin from crude human immunoglobulin samples. Recombinant Protein G has a mass of approximately 22kDa. However, its apparent mass by SDS-PAGE is nearly 34kDa.

Immobilized Protein G is most commonly used for the purification of mammalian monoclonal and polyclonal antibodies that do not bind well to Protein A. It has been reported that most mammalian immunoglobulins bind with greater affinity to Protein G than Protein A.[1] There are, however, species to which Protein A has greater affinity.[3] Protein G binds with significantly greater affinity to several immunoglobulin subclasses including human IgG_3 and rat IgG_{2a}. Unlike Protein A, Protein G does not bind to human IgM, IgD or IgA.[1]

Differences in binding characteristics between Protein A and Protein G are explained by differences in the immunoglobulin binding sites of each protein. Although the tertiary structures of these proteins are similar, their amino acid compositions differ significantly.

Inconsistency in reporting of Protein G binding characteristics occurs in the literature. One cause for this inconsistency likely results from differences in the particular source and isolation method used for the native Protein G characterized in each study. In addition, several methods have been used to assess relative binding affinity including radiolabeling experiments and ELISA techniques, the results of which are not directly comparable. Finally, significant binding differences result from different binding buffers used with Protein G. Optimal binding for most immunoglobulins to Protein G occurs in sodium acetate buffer, pH 5.0,[4] although many studies have used more neutral Tris or phosphate buffers for binding. Approximately 44% more IgG from rat serum bound to Protein G using acetate buffer, pH 5.0 (e.g., Protein G IgG Binding Buffer, Product # 21011) compared to Tris•HCl pH 7.5 buffer.

Immobilized Protein G Products

Like Immobilized Protein A already discussed, Thermo Scientific Pierce Immobilized Protein G is offered in several package sizes, columns and kit formats for your convenience in gravity-flow, spin and automated purification procedures. Our Immobilized Protein G products incorporate the recombinant form of Protein G immobilized to either crosslinked 6% beaded agarose or UltraLink Biosupport. For a more detailed description of supports, see the previous page about Immobilized Protein A Products. Both types of Immobilized Protein G use coupling chemistries that are leak-resistant and provide a matrix with minimal nonspecific binding. Both supports can be regenerated and reused multiple times when stored properly.

The Thermo Scientific Pierce Protein G Chromatography Cartridges (Product #s 89926 and 89927) are designed for fast, consistent separations using a syringe, pump or chromatography system. They are available in 1mL and 5mL sizes. Column inlet and outlet are molded with 1/16" threads and adaptors are included for coupling directly to most chromatography systems. Luer-Lok Adaptors are also provided with the columns for simple attachment to a syringe.

For the greatest convenience, choose Thermo Scientific NAb Protein G Spin Kits (Product #s 89949 and 89979), which include pre-packed columns of our Protein G Agarose, as well as binding, elution and neutralization buffers. These kits contain everything needed to isolate IgG from serum, ascites or cell culture supernatants through a fast and simple process that requires approximately 30 minutes to complete. The columns in the NAb Kits can each be regenerated a minimum of 10 times without a significant loss of binding capacity.

References
1. Bjorck, L. and Kronvall, G. (1984). *J. Immunol.* **133,** 969-974.
2. Guss, B., *et al.* (1986). *EMBO J.* **5,** 1567-1575.
3. Eliasson, M., *et al.* (1988). *J. Biol. Chem.* **263,** 4323-4327.
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Immobilized Protein G Products

Ordering Information

Product #	Description	Pkg. Size
20398	Protein G Agarose Support: Crosslinked 6% beaded agarose Capacity: 11-15mg human IgG/mL resin	2mL
20399	Protein G Agarose Support and Capacity: Same as above	10mL
20397	Protein G Agarose Support and Capacity: Same as above	25mL
89926	Pierce Chromatography Cartridges, Protein G Support and Capacity: Same as above	2 x 1mL
89927	Pierce Chromatography Cartridge, Protein G Support and Capacity: Same as above	1 x 5mL
89953	NAb Protein G Spin Columns Support and Capacity: Same as above	10 x 0.2mL
89957	NAb Protein G Spin Columns Support and Capacity: Same as above	5 x 1mL
89961	NAb Protein G Spin Columns Support and Capacity: Same as above	1 x 5mL
89949	NAb Protein G Spin Purification Kit Includes: Protein G Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer Collection Tubes	Kit 10 x 0.2mL 500mL 50mL 7mL 8 x 2mL
89979	NAb Protein G Spin Purification Kit Includes: Protein G Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer	Kit 2 x 1mL 500mL 240mL 7mL
21193	Pierce Recombinant Protein G	5mg
53125	Protein G UltraLink Resin Support: UltraLink Biosupport Capacity: > 20mg of human IgG/mL resin	2mL
53126	Protein G UltraLink Resin Support and Capacity: Same as above	10mL
53127	Protein G UltraLink Columns Support and Capacity: Same as above	2 x 2mL
45204	Protein G Spin Plate 96-well filter plate containing Protein G Agarose for IgG screening	1 plate

Immobilized Protein G Plus Products

Twice the amount of coupled Protein G per milliliter of resin.

Ordering Information

Product #	Description	Pkg. Size
22851	Protein G Plus Agarose Support: Crosslinked 6% beaded agarose Capacity: > 20mg human IgG/mL resin	2mL
22852	Protein G Plus Agarose Support and Capacity: Same as above	10mL
53128	Protein G Plus UltraLink Resin Support: UltraLink Biosupport Capacity: > 25mg human IgG/mL resin	2mL

MagnaBind Protein G Beads

Ordering Information

Product #	Description	Pkg. Size
21349	MagnaBind Protein G Beads Support: 1-4µm iron oxide particles Capacity: > 0.2mg rabbit IgG/mL beads	5mL

Protein G Coated Microplates

Ordering Information

Product #	Description	Pkg. Size
15131	Protein G, Clear 96-Well Plates Coating Volume: 100µL Blocking: SuperBlock® Blocking Buffer, 200µL Capacity: ~ 2pmol rabbit IgG/well	5 plates
15133	Protein G, Clear 8-Well Strip Plates Specifications: Same as above	5 plates
15156	Protein G, White 96-Well Plates Specifications: Same as above	5 plates
15157	Protein G, Black 96-Well Plates Specifications: Same as above	5 plates

Protein A/G

Protein A/G Beads – Quick Reference	
Protein A/G	Recombinant protein expressed in *E. coli* (50.5kDa; six IgG-binding sites)
Specificity (Table 3)	Best for polyclonal IgG from many species; poor for individual subclasses that have highest affinity for Protein A or Protein G.
Supports Offered	Crosslinked 6% beaded agarose UltraLink Biosupport
Package Formats	Resin slurries (3 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) NAb IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze

Protein A/G is a genetically engineered protein that combines the IgG binding profiles of both Protein A and Protein G. Protein A/G is a gene fusion product with a mass of 50.5kDa, designed to contain four Fc binding domains from Protein A and two from Protein G. Protein A/G is not as pH-dependent as Protein A (see page 28) but otherwise has the additive properties of Protein A and G.

Protein A/G binds to all human IgG subclasses. In addition, it binds to IgA, IgE, IgM and, to a lesser extent, IgD. Protein A/G also binds well to all mouse IgG subclasses but does not bind mouse IgA, IgM or serum albumin.[1] This makes Protein A/G an excellent tool for purification and detection of mouse monoclonal antibodies from IgG subclasses, without interference from IgA, IgM and murine serum albumin. Individual subclasses of mouse monoclonals are more likely to have a stronger affinity to the chimeric Protein A/G than to either Protein A or Protein G.[1]

Immobilized Protein A/G is an ideal choice for purification of polyclonal or monoclonal IgG antibodies whose subclasses have not been determined. Overall binding capacity is greater when pH 8.0 buffer (optimal for Protein A) is used rather than pH 5.0 buffer, which is optimal for Protein G used alone. Furthermore, Thermo Scientific Pierce Protein A Binding Buffer provides for greater binding than Tris•HCl, pH 8.0 (see description of IgG Binding and Elution Buffers on page 37).

Immobilized Protein A/G Products

Like Immobilized Protein A and G already discussed, Thermo Scientific Pierce Immobilized Protein A/G is offered in several package sizes, columns and kit formats for your convenience in gravity-flow, spin and automated purification procedures.

The Thermo Scientific Pierce Protein A/G Chromatography Cartridges (Product # 89930 and 89931) are designed for fast, consistent separations using a syringe, pump or chromatography system. They are available in 1mL and 5mL sizes. Column inlet and outlet are molded with 1/16" threads and adaptors are included for coupling directly to most chromatography systems. Luer-Lok Adaptors are also provided with the columns for simple attachment to a syringe.

For the greatest convenience, choose Thermo Scientific NAb Protein A/G Spin Kits (Product #s 89950 and 89980) which include pre-packed columns of Protein A/G Agarose, as well as binding, elution and neutralization buffers. These kits contain everything needed to isolate IgG from serum, ascites or cell culture supernatants through a fast and simple process that requires approximately 30 minutes to complete. The columns in the NAb Kits can each be regenerated a minimum of 10 times without a significant loss of binding capacity.

Reference
1. Eliasson, M., *et al.* (1988). *J. Biol. Chem.* **263,** 4323-4327

Protein A/G Coated Microplates

Ordering Information

Product #	Description	Pkg. Size
15138	Protein A/G, Clear 96-Well Plates Coating Volume: 100µL Blocking: SuperBlock Blocking Buffer, 200µL Capacity: ~ 5pmol rabbit IgG/well	5 plates

Immobilized Protein A/G Products

Ordering Information

Product #	Description	Pkg. Size
20421	Protein A/G Agarose Support: Crosslinked 6% beaded agarose Capacity: > 7mg human IgG/mL resin	3mL
20422	Protein A/G Agarose Support and Capacity: Same as above	15mL
89930	Pierce Chromatography Cartridges, Protein A/G Support and Capacity: Same as above	2 x 1mL
89931	Pierce Chromatography Cartridge, Protein A/G Support and Capacity: Same as above	1 x 5mL
89954	NAb Protein A/G Spin Columns Support and Capacity: Same as above	10 x 0.2mL
89958	NAb Protein A/G Spin Columns Support and Capacity: Same as above	5 x 1mL
89962	NAb Protein A/G Spin Column Support and Capacity: Same as above	1 x 5mL
89950	NAb Protein A/G Spin Purification Kit Includes: Protein A/G Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer Collection Tubes	Kit 10 x 0.2mL 500mL 50mL 7mL 8 x 2mL
89980	NAb Protein A/G Spin Purification Kit Includes: Protein A/G Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer	Kit 2 x 1mL 500mL 240mL 7mL
53132	Protein A/G UltraLink Resin Support: UltraLink Biosupport Capacity: > 20mg human IgG/mL resin	2mL
53133	Protein A/G UltraLink Resin Support and Capacity: Same as above	10mL
21186	Pierce Recombinant Protein A/G	5mg

Immobilized Protein A/G Plus Products

Twice the amount of coupled Protein A/G per milliliter of resin.

Ordering Information

Product #	Description	Pkg. Size
20423	Protein A/G Plus Agarose Support: Crosslinked 6% beaded agarose Capacity: > 50mg human IgG/mL resin	2mL
53135	Protein A/G Plus on UltraLink Support Support: UltraLink Biosupport Capacity: > 28mg human IgG/mL resin	2mL

Protein L

Protein L Beads — Quick Reference	
Protein L	Recombinant protein expressed in *E. coli* (35.8kDa; four IgG-binding sites)
Specificity (Table 3)	Best for human or mouse monoclonal antibodies known to have appropriate kappa light chains; poor for general-purpose (polyclonal) IgG purification
Support	Crosslinked 6% beaded agarose
Package Formats	Resin slurries (2 sizes) Chromatography Cartridges (2 sizes) Packed Spin Columns (3 sizes) NAb IgG Purification Kits (2 sizes)
Storage	4°C, do not freeze

Protein L is an immunoglobulin-binding protein (35.8kDa) that originates from the bacteria *Peptostreptococcus magnus*, but is now produced recombinantly. Unlike Protein A and Protein G, which bind primarily through Fc regions (i.e., heavy chain) of immunoglobulins, Protein L binds immunoglobulins through interactions with their light chains. Since no part of the heavy chain is involved in the binding interaction, Protein L binds a wider range of Ig classes than Protein A or G. Protein L will bind to representatives of all classes of Ig including IgG, IgM, IgA, IgE and IgD. Single-chain variable fragments (ScFv) and Fab fragments can also be bound by Protein L.

Despite this wide-ranging binding capability with respect to Ig classes (which are defined by heavy chain type), Protein L is not a universal immunoglobulin-binding protein. Binding of Protein L to immunoglobulins is restricted to those containing kappa light chains (i.e., κ chain of the V_L domain).[1] In humans and mice, kappa (κ) light chains predominate. The remaining immunoglobulins have lambda (λ) light chains. Furthermore, Protein L is effective in binding only certain subtypes of kappa light chains. For example, it binds human Vκl, VκIII and VκIV subtypes but does not bind the VκII subtype. Binding of mouse immunoglobulins is restricted to those having Vκl light chains.[1]

Given these specific requirements for effective binding, immobilized Protein L is not appropriate for general polyclonal antibody purification from serum, which contains a mixture of immunoglobulins having different types of light chains. The main application for immobilized Protein L is purification of monoclonal antibodies from ascites or culture supernatant that are known to have the Vκl light chain. Protein L is extremely useful for this specific application because it does not bind bovine immunoglobulins, which are present in the media serum supplement. Also, in contrast to Protein A and G, Protein L is very effective at binding IgM. Although it binds to the Fab portion of the immunoglobulin monomer, Protein L does not interfere with the antigen-binding site of the antibody. Therefore, Protein L potentially can be used in immunoprecipitation (IP) procedures.

Immobilized Protein L Products

Like Immobilized Protein A and G already discussed, Thermo Scientific Pierce Immobilized Protein L is offered in several package sizes, columns and kit formats for your convenience in gravity-flow, spin and automated purification procedures.

The Thermo Scientific Pierce Protein L Chromatography Cartridges (Product #s 89928 and 89929) are designed for fast, consistent separations using a syringe, pump or chromatography system. They are available in 1mL and 5mL sizes. Column inlet and outlet are molded with 1/16" threads and adaptors are included for coupling directly to most chromatography systems. Luer-Lok Adaptors are also provided with the columns for simple attachment to a syringe.

For the greatest convenience, choose Thermo Scientific NAb Protein L Spin Kits (Product #s 89951 and 89981) which include pre-packed columns of Protein L Agarose, as well as binding, elution and neutralization buffers.

Reference
1. Nilson, B., *et al.* (1992). *J. Biol. Chem.* **267,** 2234-2238.

Immobilized Protein L Products

Ordering Information

Product #	Description	Pkg. Size
20510	**Protein L Agarose** Support: Crosslinked 6% beaded agarose Capacity: 5-10mg human IgG/mL resin	2mL
20512	**Protein L Agarose** Support and Capacity: Same as above	10mL
89928	**Pierce Chromatography Cartridges, Protein L** Support and Capacity: Same as above	2 x 1mL
89929	**Pierce Chromatography Cartridge, Protein L** Support and Capacity: Same as above	1 x 5mL
89955	**NAb Protein L Spin Columns** Support and Capacity: Same as above	10 x 0.2mL
89959	**NAb Protein L Spin Columns** Support and Capacity: Same as above	5 x 1mL
89963	**NAb Protein L Spin Column** Support and Capacity: Same as above	1 x 5mL
89951	**NAb Protein L Spin Purification Kit** Includes: Protein L Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer Collection Tubes	Kit 10 x 0.2mL 500mL 50mL 7mL 8 x 2mL
89981	**NAb Protein L Spin Purification Kit** Includes: Protein L Spin Columns PBS Binding Buffer IgG Elution Buffer Neutralization Buffer	Kit 2 x 1mL 500mL 240mL 7mL
21189	**Pierce Recombinant Protein L**	1mg

Immobilized Protein L Plus Products

Twice the amount of coupled Protein L per milliliter of resin.

Ordering Information

Product #	Description	Pkg. Size
20520	**Protein L Plus Agarose** Support: Crosslinked 6% beaded agarose Capacity: > 10-20mg human IgG/mL resin	2mL

Protein L Coated Microplates

Ordering Information

Product #	Description	Pkg. Size
15190	**Protein L, Clear 96-Well Plates** Coating Volume: 100µL Blocking: SuperBlock Blocking Buffer, 200µL	5 plates

IgG Binding and Elution Buffers for Protein A, G, A/G and L

Binding and Elution Steps in Affinity Purification

Affinity purification procedures involving interaction of an antibody with its antigen generally use binding buffers at physiologic pH and ionic strength. However, many antibody purification methods do not use the antibody-antigen interaction; rather, they involve binding of antibodies by immobilized ligands that are not the antigen. In such cases, optimal binding conditions are determined by the unique properties of the antibody-ligand interaction, which may be different from physiologic pH and ionic strength.

Once the binding interaction occurs (i.e., the antibody is "captured" by the immobilized ligand), the support is washed with additional buffer to remove nonbound components of the sample. Finally, elution buffer is added to break the binding interaction and release the target molecule, which is then collected in its purified form. Elution buffer can dissociate binding partners by extremes of pH (low or high), high salt (ionic strength), the use of detergents or chaotropic agents that denature one or both of the molecules, removal of a binding factor, or competition with a counter ligand. In most cases, subsequent dialysis or desalting is required to exchange the purified protein from elution buffer into a more suitable buffer for storage or use.

Thermo Scientific Pierce IgG Binding and Elution Buffers have been optimized to provide the highest possible efficiency of IgG binding and elution using immobilized Protein A, Protein G and Protein A/G. Use of other buffer formulations may significantly alter not only the binding capacity but also the volumes of wash buffer required to ensure good purification.

General Binding and Elution Buffers for Protein A, G, A/G and L

Although Protein A, G and A/G bind immunoglobulins adequately at physiologic pH and ionic strength (as with phosphate-buffered saline, pH 7.2), optimal binding conditions are different for each protein. For this reason, separate IgG Binding Buffers are available for use with each immobilized "alphabet protein" product. All our buffers have long shelf lives and are premixed for maximum ease of use.

The Thermo Scientific Pierce Protein A IgG Binding Buffer is a unique, phosphate-based formulation (pH 8.0) that achieves maximum binding capacity of IgG to immobilized Protein A. Overall IgG binding capacity is increased with this buffer relative to traditional binding buffers (see Table 4). Most notably, the otherwise weak binding of mouse IgG_1 is greatly improved.

Thermo Scientific Pierce Protein G IgG Binding Buffer uses sodium acetate (pH 5.0) to obtain the highest possible binding capacity of IgG to immobilized Protein G. The binding buffer for Protein A/G is similar to our Protein A IgG Binding Buffer. The optimal binding with Protein L occurs at pH 7.5; NAb Protein L Kits use phosphate-buffered saline (PBS) as the binding buffer.

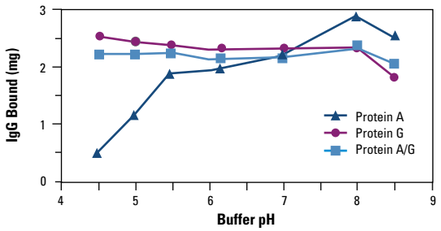
Generally, a Pierce Binding Buffer is used by combining it 1:1 (v/v) with clarified serum or ascites fluid. To avoid dilution, a sample can be dialyzed into the recommended buffer. Purity of the samples affects the total binding capacity of Protein A, G and A/G; total immunoglobulin binding capacities are higher for purified and concentrated antibodies than for crude serum or dilute samples.

Elution of antibodies that are bound to alphabet proteins, regardless of the binding buffer used, is most generally accomplished using 0.1 M glycine•HCl (pH 2-3) or other low pH buffer. In the vast majority of cases, this condition breaks affinity interactions without damaging either the immobilized protein (allowing the affinity column to be re-used) or the antibody. Our IgG Elution Buffer uses this acidic (pH 2.8) condition. With this buffer, elution of IgG is usually sharp and complete. For example, nearly all bound IgG will elute in 3mL of buffer from a 1mL column of Protein A.

Although brief exposure of antibody to acidic elution buffer usually is not harmful, it is advisable to neutralize the eluate as soon as possible after its recovery to minimize the possibility of degradation. Our IgG Elution Buffer can be neutralized easily by adding 1/10th volume of 1 M Tris•HCl, pH 7.5-9.0. Although long-term storage of the purified antibody in the neutralized buffer is possible, it is common practice to dialyze or desalt into a buffer that is known to be suitable for storage.

Table 4. Binding capacities with different Thermo Scientific Buffers expressed as mg of IgG bound per 2mL of resin.

	Immobilized Protein A		Immobilized Protein G		Immobilized Protein A/G	
Serum Sample	0.1 M Tris•HCl pH 8.0	Pierce Protein A Binding Buffer	0.1 M Tris•HCl pH 8.0	Pierce Protein G Binding Buffer	0.1 M Tris•HCl pH 8.0	Pierce Protein A Binding Buffer
Rabbit	17.81	33.19	21.51	27.75	13.89	19.61
Sheep	2.15	10.64	25.53	33.33	9.83	15.71
Bovine	6.16	22.76	31.72	48.10	15.13	22.06
Mouse	5.25	7.15	5.65	15.05	4.32	11.49
Rat	4.99	8.30	8.43	11.80	5.20	6.66
Horse	6.25	16.50	36.19	21.46	14.88	17.12
Dog	35.77	22.27	13.38	20.55	21.96	24.60
Chicken	0.91	1.21	1.63	7.27	1.21	4.10
Pig	29.61	24.83	21.25	27.51	19.24	29.48
Human	19.88	25.53	11.68	23.59	9.92	17.67



Comparison of the binding characteristics of mouse IgG at various buffer pH levels.

Gentle Ag/Ab Elution Buffer

Some antibodies are extremely labile and irreversibly denature in the acidic conditions of the default Pierce IgG Elution Buffer. Our Gentle Ag/Ab Elution Buffer is available for such situations. This near-neutral (pH 6.55) buffer dissociates affinity-bound immunoglobulins by ionic strength rather than by low pH. While being much less likely to degrade an antibody, it still retains excellent elution properties.

Our researchers have tested the effect of exposure to our Gentle Elution Buffer on monoclonal antibody activity. In one experiment, three mouse monoclonals were incubated overnight in the Gentle Elution Buffer and then desalted. When analyzed in an ELISA system, all three monoclonals retained full antigen-binding capability as compared to untreated controls.

The Gentle Elution Buffer does not require neutralization and is directly compatible with borate, citrate and acetate buffers, including our Protein G IgG Binding Buffer. However, the Gentle Elution Buffer is not directly compatible with phosphate-containing buffers, including our Protein A IgG Binding Buffer, with which it will form an insoluble precipitate. For this reason, our Gentle Ag/Ab Binding Buffer, pH 8.0 is offered as a substitute for use with Protein A.

Mouse IgG_1 Mild Elution Buffer

A unique opportunity exists in Protein A with its weaker binding affinity to mouse IgG_1 compared to other mouse IgG subclasses. After binding total mouse IgG to immobilized Protein A using our Protein A IgG Binding Buffer, Thermo Scientific Pierce Mouse IgG_1 Mild Elution Buffer can be used to selectively elute IgG_1 without affecting the bound state of other IgG subclasses.

The buffer has a mild pH (6.0-6.1) to retain better biological activity in both the recovered antibody and the immobilized Protein A. Neutralization or desalting of the collected IgG_1 is not necessary to retain activity. This advantage is especially important when isolating potentially fragile monoclonal IgG_1 antibodies. Because the majority of mouse monoclonals are of the IgG_1 subclass, this buffer has many applications in the production of monoclonal antibodies.

After eluting the IgG_1, other bound IgGs can be eluted using standard IgG Elution Buffer. Our Protein A Binding Buffer and both IgG and IgG_1 Mild Elution Buffers are available as a kit. The system enables quick, clean and mild isolation of mouse IgG_1 from serum, ascites or hybridoma culture supernatant.

Ordering Information

Product #	Description	Highlights	Pkg. Size
54200	Protein A/G IgG Binding Buffer	• Ensures maximum recovery of IgG from immobilized Protein A/G	240mL
21001 21007	Protein A IgG Binding Buffer	• High-yield isolation of Mouse IgG_1 using Protein A columns • Premixed and easy to use	1L 3.75L
21019 21011	Protein G IgG Binding Buffer	• Ensures maximum recovery of IgG from immobilized Protein G	1L 3.75L
21004 21009	IgG Elution Buffer	• High-yield isolation of IgG from Immobilized Protein A and Protein G	1L 3.75L
21020 21012	Gentle Ag/Ab Binding Buffer pH 8.0	• Specially formulated and prefiltered • Eliminates use of harsh acidic elution conditions	1 L 3.75L
21030 21027 21013	Gentle Ag/Ab Elution Buffer pH 6.6	• Specially formulated for neutral elutions • Not compatible with phosphate buffers	100mL 500mL 3.75L
21016	IgM Binding Buffer	• Specially formulated for optimal binding of mouse IgM	800mL
21017	IgM Elution Buffer	• Specially formulated for optimal recovery of mouse IgM	500mL
21018	MBP Column Preparation Buffer	• Specially formulated for use with Immobilized MBP and IgM Purification Kit	50mL
21034	Mouse IgG_1 Mild Elution Buffer	• Separate IgG_1 from other IgG subclasses	500mL
21033	Mouse IgG_1 Mild Binding and Elution Buffer Kit Includes: Pierce Protein A IgG Binding Buffer Mouse IgG_1 Mild Elution Buffer Pierce IgG Elution Buffer	• Complete kit to allow mouse IgG_1 to be separated from other mouse IgG subclasses	Kit 1L 500mL 1L

Melon™ Gel Purification Products

Thermo Scientific Melon Gel Products provide an exciting new approach to purifying monoclonal and polyclonal antibodies from serum, tissue culture supernatant and ascites fluid. Melon Gel works with antibodies from a variety of species and subclasses, many of which do not purify efficiently with Protein A or Protein G. Because Melon Gel is not a bind-and-release support, it is extremely fast and gentle to your antibodies, resulting in antibody preparations of high purity and high activity!

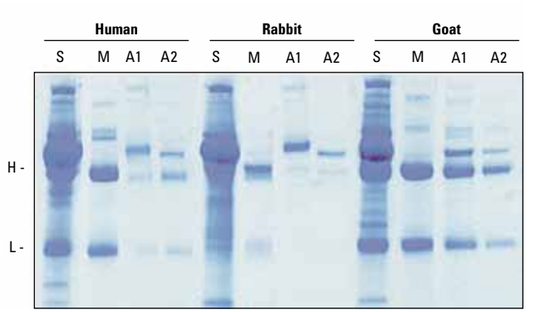
How does it work?

Melon Gel contains a proprietary ligand that retains most protein found in serum, ascites and culture supernatants, while allowing IgG to pass through the support and be collected in the flow-through fraction. The resulting recovery and purity of the IgG isolated by this method rivals that obtained from the same samples using bind-and-release supports such as Protein A or Protein G.

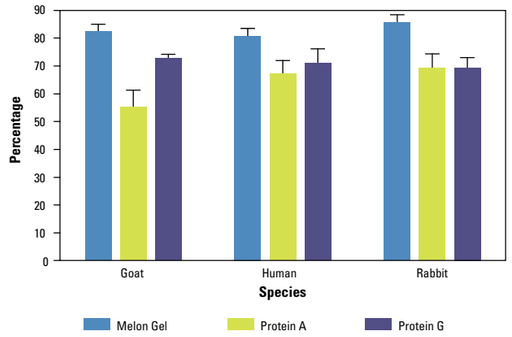
Highlights:

- Simple, one-step protocol – no tedious binding, washing, and multiple elution steps
- Rapid purification – purifies antibodies from serum four to six times faster than Protein A or G methods
- High recovery and purity – antibodies from many species are recovered with greater than 90% yield and greater than 80% purity
- Robust purification – works with a wide range of antibodies including many that do not purify well on Protein A or Protein G
- Gentle purification – no harsh elution conditions means antibodies retain more activity
- Reusable support – Melon Gel Support can be used for multiple antibody purifications
- Available in various formats – spin columns, purification kits and chromatography cartridges for antibody purification from serum, ascites and culture supernatant

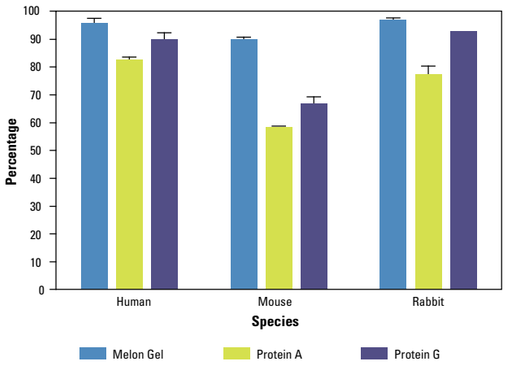
Several kits and package sizes of Melon Gel Resin are available. All sizes use the same formulation of Melon Gel Resin, Purification Buffer and Regenerant Solution but have slightly different protocols based on the most common application for each package size. Components of any package size can be used at an appropriate scale with any one of the procedures.



Thermo Scientific Melon Gel efficiently purifies IgG from human, rabbit and goat serum. Starting serum samples and resulting purification products were electrophoresed by SDS-PAGE and stained with Thermo Scientific GelCode Blue Stain. S = serum sample, M = Melon Gel-purified product, A1 and A2 = successive elution fractions from Protein A purification. H and L denote antibody heavy and light chain bands in the reducing gel. Similar results were obtained when comparing against Protein G purification.



Thermo Scientific Melon Gel provides better purity than Protein A and G. Percent purity was determined by purifying IgG from goat, human and rabbit serum using Melon Gel, Protein A and Protein G. The products were separated by SDS-PAGE. Purity was calculated by determining the intensities of all bands via fluorescence scanning and densitometry.

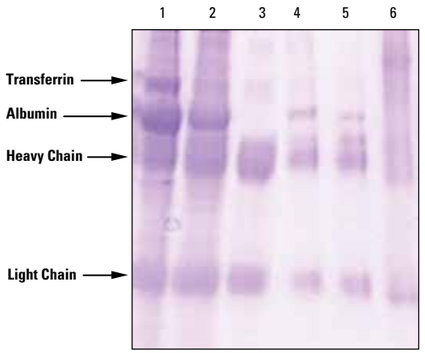


Thermo Scientific Melon Gel provides better recovery than Protein A and G. Percent recovery was determined by applying human, mouse and rabbit IgG to Melon Gel, Protein A and Protein G, and then comparing the absorbance at 280nm of the original samples against that of the purified sample.

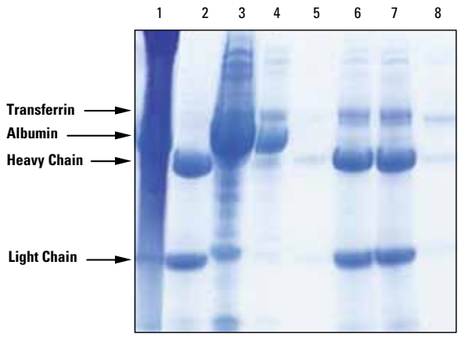
IgG purification performance of the Thermo Scientific Melon Gel System, Protein A and Protein G.

Source	Melon Gel	Protein A	Protein G
Human	H	H	H
Mouse	H	H	H
Rabbit	H	H	H
Rat	H	L	M
Goat	H	L	M
Cow	M	L	H
Sheep	M	L	H
Horse	H	L	H
Guinea Pig	H	H	L
Pig	H	H	L
Chicken	N	N	N
Hamster	H	M	M
Donkey	H	M	H

H = high recovery, M = medium recovery, L = low recover, N = no recovery



Thermo Scientific Melon Gel offers better recovery and purity from cell culture supernatant than Protein G. IgG was purified from cell culture supernatant containing 10% fetal bovine serum (FBS), resolved by SDS-PAGE and detected with Thermo Scientific Imperial Protein Stain (Product # 24615). **Lane 1.** Original cell culture supernatant, **Lane 2**. IgG after precipitation with Saturated Ammonium Sulfate (Product # 45216), **Lane 3**. Melon Gel-purified IgG and **Lane 4-6**. Protein G-purified IgG.



Thermo Scientific Melon Gel offers better recovery and purity from ascites fluid than Protein G. IgG_1 was purified from ascites fluid, resolved by SDS-PAGE and detected with GelCode™ Blue Stain Reagent (Product # 24590). **Lane 1**. Original ascites fluid, **Lane 2**. Treated with Ascites Conditioning Reagent (Product # 45219) and purified with Melon Gel, **Lane 3**. Protein G column flow-through, **Lanes 4-5**. Protein G column washes and **Lane 6-8**. Protein G column elutions. NOTE: The Protein G-purified sample is contaminated with transferrin (Lanes 6-8). The Melon Gel-purified sample is not (Lane 2).

Ordering Information

Product #	Description	Pkg. Size
45206	**Melon Gel IgG Spin Purification Kit** *Sufficient to purify up to 25mg of IgG from serum.* Includes: Melon Gel Melon Gel Purification Buffer Spin Columns Collection Tubes	Kit 3mL 100mL 27
45212	**Melon Gel IgG Spin Purification Kit** *Sufficient to purify up to 2g of IgG from serum.* Includes: Melon Gel Support Melon Gel Purification Buffer Melon Gel Regenerant (dry mix; reconstitute to 1L)	Kit 25mL 100X, dry mix
45214	**Melon Gel Monoclonal IgG Purification Kit** *Sufficient to purify IgG from up to 1L of cell culture supernatant or up to 200mL of ascites fluid.* Includes: Melon Gel Support Melon Gel Purification Buffer Melon Gel Regenerant	Kit 200mL 100X, dry mix 5X, dry mix
45216	**Saturated Ammonium Sulfate Solution** *For use with the Melon Gel Monoclonal IgG Purification Kit and Melon Gel IgG Purification Kits for Sera or as a general-purpose salting-out agent for protein-preparation applications.*	1L
45219	**Ascites Conditioning Reagent** *For use with the Melon Gel Monoclonal IgG Purification Kit.* *Use of this reagent is described in the instructions provided with Product # 45214.*	5mL
89932	**Pierce Chromatography Cartridges, Melon Gel**	2 x 1
89933	**Pierce Chromatography Cartridges, Melon Gel**	1 x 5
45208	**Melon Gel Spin Kit Plate** 96-well filter plate for IgG screening	2 plates
89972	**Melon Gel Purification Buffer**	1 pack
89973	**Melon Gel Regenerant**	1 pack

Thiophilic Gel Antibody Purification

Thiophilic adsorption is a low-cost, efficient alternative to ammonium sulfate precipitation for immunoglobulin purification from crude samples. Ammonium sulfate precipitation must be followed by several additional steps to completely remove contaminants in crude samples. Thiophilic adsorption is a simple, rapid, one-step method for antibody purification from serum, ascites or tissue culture supernatant.

Thiophilic adsorption is a highly selective type of lyotropic salt-promoted protein:ligand interaction phenomenon that has been studied extensively by Porath and co-workers and other researchers.[1] This interaction is termed thiophilic because it distinguishes proteins that recognize a sulfone group in close proximity to a thioether. Thiophilic adsorption incorporates properties of both hydrophobic and hydrophilic adsorption. However, in contrast to strictly hydrophobic systems, thiophilic adsorption is not strongly promoted by high concentrations of sodium chloride. Instead, thiophilic adsorption is promoted by increased concentrations of water-interacting, non-chaotropic salts such as potassium and ammonium sulfate.

Thermo Scientific Pierce Thiophilic Adsorbent is 6% beaded agarose modified to contain simple sulfone/ thioether groups (see structure at right). Our Thiophilic Adsorbent has a high binding capacity (20mg of immunoglobulin per mL of resin) and broad specificity toward immunoglobulins derived from various animal species. Notably, thiophilic adsorption is one of few methods available for purification of IgY from chicken (see also subsequent discussion of IgY purification). Among human serum proteins, immunoglobulins and α2-macroglobulins are preferentially bound by our Thiophilic Adsorbent.[2]

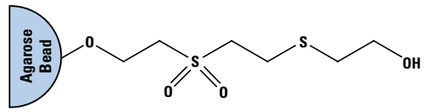
Purification using Pierce Thiophilic Adsorbent results in good protein recovery with excellent preservation of antibody activity. Sample preparation requires the addition of 0.5M potassium sulfate to the serum, ascites or culture fluid. Greater specificity for immunoglobulins is obtained if the sample is buffered at pH 8.0. The gentle elution conditions (e.g., 50mM sodium phosphate, pH 7-8) yield concentrated, essentially salt-free, highly purified immunoglobulins at near neutral pH.

After use, our Thiophilic Adsorbent can be regenerated by treatment with guanidine•HCl. Our data indicate that the Adsorbent column can be used at least 10 times without significant loss of binding capacity.

Our Thiophilic Purification Kit includes 4 x 3mL prepacked columns of Pierce Thiophilic Adsorbent, binding and elution buffers, column storage buffer, and guanidine•HCl for use in column regeneration. This simple, one-step method eliminates the need for post-treatment of the sample before storage or subsequent conjugation to enzymes for use in immunoassays.

Suggested applications:

- Efficient and selective isolation of immunoglobulins from human serum under mild conditions[1]
- Convenient and fast method for purification of mouse monoclonals from the culture media of cloned cells or from ascites fluid[2]
- Selective removal of immunoglobulins from fetal calf serum – useful for cell culture in monoclonal antibody production[3]
- Rapid, straightforward procedure yielding essentially pure immunoglobulins from crude rabbit serum[4]
- Purification of IgY from chicken[5]
- Large-scale purification for biotechnology applications



Structure of Thermo Scientific Pierce Thiophilic Adsorbent.

References
1. Porath, J., *et al.* (1985). *FEBS Lett.* **185,** 306-310.
2. Belew, M., *et al.* (1987). *J. Immunol. Method* **102,** 173-182.
3. Hutchens, T.W. and Porath, J. (1987). *Biochemistry* **26,** 7199-7204.
4. Lihme, A. and Heegaard, P.M.H. (1990). *Anal. Biochem.* **192,** 64-69.
5. Unpublished internal documents.

Thiophilic Adsorbent and Purification Kit

Economical purification of mouse antibodies from ascites fluid.

Highlights:

- Binds to Fab and F(ab´)$_2$ fragments
- Binds to ScFv[1]
- High-capacity (20mg/mL), good protein recovery and retention of antibody function
- Broad specificity toward immunoglobulins derived from various animal species (see Table 5)
- Binds chicken IgY (also IgG)
- Simple, rapid, one-step purification for monoclonal antibodies from ascites; easy to scale up
- Used to enrich the immunoglobulin fraction from serum or tissue culture supernatant
- Efficient alternative to ammonium sulfate precipitation for enriching antibodies from crude samples
- Gentle elution conditions yield concentrated, salt-free immunoglobulin at near neutral pH
- High degree of purity

Table 5. Binding characteristics of Thermo Scientific Pierce Thiophilic Adsorbent.

Species	Total A_{280} Bound from 1mL Serum	% Purity by HPLC
Human	4.8	70
Mouse	8.6	63
Mouse IgG_1	11.6	92
Mouse IgG_{2a}	9.3	88
Mouse IgG_{2b}	9.8	97
Mouse IgG_3	10.7	94
Rat	13.0	79
Bovine	17.9	90
Calf	11.1	89
Chicken	5.2	76
Dog	12.2	91
Goat	17.3	92
Guinea Pig	11.1	71
Horse	13.0	93
Pig	21.1	90
Rabbit	6.7	84
Sheep	12.3	89

References

1. Schulze, R.A., *et al.* (1994). *Anal. Biochem.* **220,** 212-214.
Palmer, D.A., *et al.* (1994). *Anal. Biochem.* **222,** 281-283.
Porath, J., *et al.* (1985). *FEBS Lett.* **185,** 306-310.
Hutchens, T.W. and Porath, J. (1986). *Anal. Biochem.* **159,** 217-226.
Belew, M., *et al.* (1987). *J. Immunol. Method* **102,** 173-182.
Hutchens, T.W. and Porath, J. (1987). *Biochemistry* **26,** 7199-7204.
Nopper, B., *et al.* (1989). *Anal. Biochem.* **180,** 66-71.
Harsay, E. and Schekman, R. (2002). *J. Cell Biol.* **156**(2), 271-85.
Koustova, E. *et al.* (2001). *J. Clin. Invest.* **107(6),** 737-44.
Suh, J.S., *et al.* (1998). *Blood.* **91(3),** 916-22.

Ordering Information

Product #	Description	Pkg. Size
20500	Pierce Thiophilic Adsorbent	10mL
44916	Pierce Thiophilic Purification Kit	Kit
	Includes: Thiophilic Adsorbent Columns	4 x 3mL
	Binding Buffer	1,000mL
	Elution Buffer	1,000mL
	Column Storage Buffer (2X)	100mL
	Guanidine•HCl Crystals	230g
	Column Extenders	

IgM Purification

Structure of IgM

IgM is a high molecular mass glycoprotein (900-950kDa) with a carbohydrate content of approximately 12%. This antibody is found at concentrations of 0.5-2mg/mL in serum.[1] *In vivo* IgM has a half life of five days, and its catabolism is two- to three-fold greater than that of IgG.

In the sera of mammals, birds and reptiles, IgM has a pentameric structure. However, mouse and human IgM structures differ in the location of disulfide bridges that link monomers together to form the pentamer (Figure 2).[2] Disulfides are arranged in series in mouse IgM and in parallel in human IgM.

Challenges to IgM Purification

Protein A binds IgM poorly, in part because binding sites on the Fc region of the monomers are sterically hindered by the pentameric structure of IgM. Until recently, no readily available affinity chromatography product existed for one-step IgM purification. Standard methods for IgM purification generally are multi-step, tedious processes or they are not effective for removing all of the major impurities present in IgM samples.[3]

Traditionally, IgM was purified by ammonium sulfate precipitation followed by gel filtration, ion exchange chromatography or zone electrophoresis.[4] Other methods that have been used include use of DEAE cellulose,[5] immobilized DNA[6] and a combination of ammonium sulfate precipitation and subsequent removal of IgG with Protein A or G.[3]

Nethery, *et al.* developed an IgM affinity purification method using C1q, a 439kDa complement component that recognizes carbohydrate on cell surfaces.[7] This temperature-dependent binding method yielded relatively pure IgM. However, co-purification of IgG was a problem, and C1q is expensive and difficult to purify.

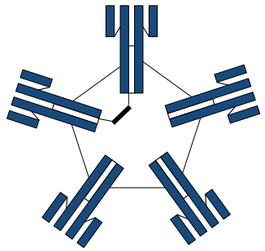
Immobilized Mannan Binding Protein

To develop an effective affinity matrix, our scientists examined C1q and another similarly structured protein, mannan binding protein (MBP). Serum MBP, like C1q, is capable of initiating carbohydrate-mediated complement activation. MBP is a mannose and *N*-acetylglucosamine-specific lectin found in mammalian sera, and it has considerable structural homology to C1q.[8] MBP subunits are identical, each with molecular mass of approximately 31kDa (C1q has six each of three different polypeptide subunits of molecular mass 24-28kDa). Studies in our labs show that MBP does not bind F(ab´)$_2$ and Fab.

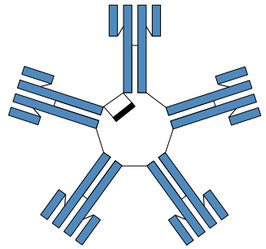
We have developed an easy-to-use Thermo Scientific Pierce Immobilized Mannan Binding Protein and Buffer System to purify IgM. It is most effective for purifying mouse IgM from ascites. Purified IgM can be obtained from a single pass over the affinity column. Human IgM will bind to the support, albeit with slightly lower capacity, and yield a product at least 88% pure as assessed by HPLC. The purification of IgM from other species and mouse serum has not yet been optimized.

IgM purification with Pierce Immobilized Mannan Binding Protein is temperature- and calcium-dependent. Binding and washing steps are performed at 4°C in 10mM Tris•HCl (pH 7.4) buffer containing sodium chloride and 20mM calcium chloride. Elution is made at room temperature in a similar Tris buffer, except that it contains EDTA and is devoid of calcium chloride. An Immobilized MBP Column can be regenerated at least 10 times with no apparent loss of binding capacity.

Immobilized MBP is available in both beaded agarose and UltraLink Biosupport formats. Binding, elution and column preparation buffers are also available. The IgM Purification Kit contains sufficient buffers to perform 10 purifications using a 5mL column of Immobilized MBP. The kit is easy to use and yields 90% pure mouse IgM (from ascites) with a very simple protocol.



Human IgM



Mouse IgM

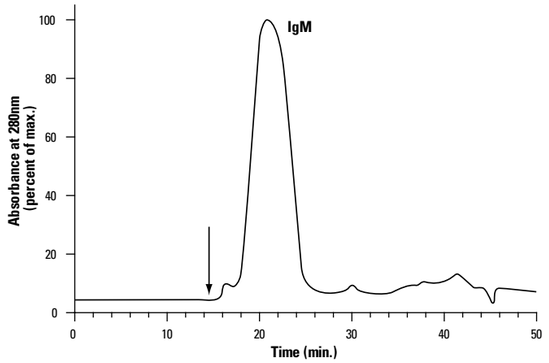
Figure 2. Structure of IgM, adapted from Matthew and Reichardt.[8]

References

1. Milstein, C., *et al.* (1975). *Biochem. J.* **151,** 615-624.
2. Coppola, G., *et al.* (1989). *J. Chromatogr.* **476,** 269-290.
3. Fahey, J. and Terry, E. (1967). Handbook of Experimental Immunology, Chapter 8. D.M. Weir, Ed. Blackwell, Oxford, U.K.
4. Cambier, J. and Butler F. (1974). *Prep. Biochem.* **4(1),** 31-46.
5. Abdullah, M., *et al.* (1985). *J. Chromatogr.* **347,** 129-136.
6. Nethery, A., *et al.* (1990). *J. Immunol. Method* **126,** 57-60.
7. Ohta, M., *et al.* (1990). *J. Biol. Chem.* **264,** 1980-1984.
8. Matthew, W. and Reichardt, L. (1982). *J. Immunol. Method* **50,** 239-253.

Immobilized MBP and IgM Purification Kit

Easy IgM purification with guaranteed 88% pure mouse IgM!



Demonstration of the high purity of MBP-purified IgM from mouse ascites. The bound material from mouse ascites was eluted from the 5mL MBP column as described in the Standard Protocol. The highest 280nm absorbing fraction from the elution was chromatographed using the conditions described in the instructions.

References
Nethery, A., *et al.* (1990). *J. Immunol. Method* **126,** 57-60.
Ohta, M., *et al.* (1990). *J. Biol. Chem.* **265,** 1980-1984.
Nevens, J.R., *et al.* (1992). *J. Chromatogr.* **597,** 247-256.

Ordering Information

Product #	Description	Pkg. Size
22212	**Immobilized Mannan Binding Protein** Capacity: ~1mg IgM/mL of resin	10mL
44897	**IgM Purification Kit** Includes: Immobilized MBP Column IgM Binding Buffer IgM Elution Buffer MBP Column Preparation Buffer Column Extender	Kit 5mL 800mL 500mL 50mL
21016	**IgM Binding Buffer**	800mL
21017	**IgM Elution Buffer**	500mL
21018	**MBP Column Preparation Buffer**	50mL
53123	**UltraLink Immobilized Mannan Binding Protein** Capacity: > 0.75mg IgM/mL of resin	5mL

IgA Purification

Human IgA Purification

Jacalin is an α-D-galactose binding lectin extracted from jackfruit seeds (*Artocarpus integrifolia*). The lectin is a glycoprotein of approximately 40kDa composed of four identical subunits. Jacalin immobilized on supports such as agarose has been useful for the purification of human serum or secretory IgA_1. IgA can be separated from human IgG and IgM in human serum or colostrum.[1] IgD is reported to bind to jacalin.[2] Immobilized jacalin is also useful for removing contaminating IgA from IgG samples.

Binding of IgA to immobilized jacalin occurs at physiologic pH and ionic strength, as in phosphate buffered saline (PBS). Elution of bound IgA occurs with competitor ligand (e.g., 0.1 M melibiose or 0.1 M α-D-galactose) in PBS. We offer immobilized jacalin on crosslinked 6% agarose.

References
1. Roque-Barreira, M.C. and Campos-Neto, A. (1985). *J. Immunol. Method* **134(30),** 1740-1743.
2. Aucouturier, P., *et al.* (1987). *Mol. Immunol.* **24(5),** 503-511.

Immobilized Jacalin

Ideal for human IgA purification.

Highlights:

- Ideal for preparing human IgA that is free of contaminating IgG
- Found to bind human IgA_1, but not human IgA_2 – useful for separating the two subclasses

References
Kumar, G.S., *et al.* (1982). *J. Biosci.* **4,** 257-261.
Roque-Barreira, M.C. and Campos-Neto, A. (1985). *J. Immunol.* **134,** 1740-1743.
Mestecky, J., *et al.* (1971). *J. Immunol.* **107,** 605-607.
Van Kamp, G.J. (1979). *J. Immunol. Method* **27,** 301-305.
Kondoh, H., *et al.* (1986). *J. Immunol. Method* **88,** 171-173.

Ordering Information

Product #	Description	Pkg. Size
20395	**Immobilized Jacalin** Capacity: 1-3mg human IgA/mL of resin Support: Crosslinked 6% beaded agarose Loading: 4.5mg of jacalin/mL of resin	5mL

Chicken IgY Purification

Properties of IgY

Chickens produce a unique immunoglobulin molecule called IgY. There are several advantages to production and use of IgY over mammalian immunoglobulins. With regard to production, raising and immunizing chickens is relatively simple, chickens are more likely to produce an immune response to conserved mammalian protein antigens, and chickens produce 15- to 20-times more antibody than rabbits.

Most importantly, IgY is naturally packaged at high concentrations in egg yolks, making repeated collection of antibody from immunized hens noninvasive. A single egg yolk from an immunized chicken contains approximately 300mg of IgY. Whole eggs or separated egg yolks can be collected and stored frozen for later extraction of antibody.

Other advantages of IgY for use in immunoassays are that it does not bind rheumatoid factor or other anti-mammalian IgGs, does not activate complement, and generally has much lower probability of nonspecific binding to mammalian tissues and extracts.

IgY Purification Methods

One challenge with regard to IgY is that it can be difficult to purify. Protein A, Protein G and other Fc-binding proteins do not bind IgY.

Pierce Thiophilic Adsorbent (see page 42) enables moderate yields of fairly pure IgY from serum and other fluids. However, complete procedures for our Thiophilic Adsorbent have not been developed for use with egg yolks, which have very high lipid concentrations.

Thermo Scientific Pierce Chicken IgY Purification Kits were specifically developed for efficient purification of IgY from egg yolks. After separating an intact yolk from egg white using an egg separator, Thermo Scientific Pierce Delipidation Reagent is added to separate the proteins from lipid. The delipidation reagent can also be used to store an egg yolk in the freezer for up to one year. After delipidation, the protein-containing sample fraction is mixed with Thermo Scientific Pierce IgY Precipitation Reagent to create a relatively pure IgY precipitate that is recovered by centrifugation.

Routinely, 80-120mg of high purity (> 85%), intact IgY can be obtained per egg using the Pierce IgY Purification Kit.

References
1. Cassidy, P.B., *et al.* (2006). *Carcinogenesis.* **27,** 2538-2549.
2. Kamiya, Y., *et al.* (2005).*J. Biol. Chem.* **280,** 37178-37182.
3. Kantardzhieva, A. *et al.* (2005). *Retinal Cell Biol.* **46,** 2192-2201.

Chicken IgY Purification Kit

Purifies 100mg of chicken IgY with higher purity than ever before!

Highlights:

- **More for your money** – purifies twice the amount of IgY as the leading competitor's kit with a lower cost-per-mg of IgY purified
- **Higher purity** – 85-95% by SDS-PAGE analysis (see Figure 3)
- **Ease-of-use** – the simple precipitation method works without affinity columns
- **Flexibility** – eggs can be stored in buffer and purified at a later date
- **Convenience** – use eggs directly out of the refrigerator; no need to wait for them to warm up

Ordering Information

Product #	Description	Pkg. Size
44918	**Chicken IgY Purification Kit** *Sufficient reagents to purify 5 egg yolks.* Includes: Delipidation Reagent IgY Precipitation Reagent Egg Separator	Kit 500mL 500mL 1
44922	**Chicken IgY Purification Kit** *Sufficient reagents to purify 25 egg yolks.*	Kit
21055	**Delipidation Reagent**	500mL
21057	**IgY Precipitation Reagent**	500mL

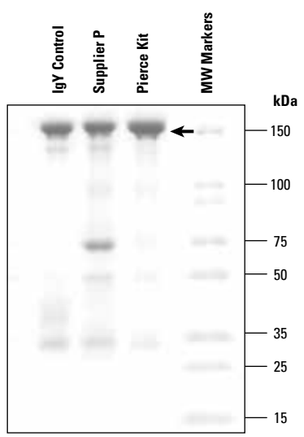


Figure 3. SDS-PAGE analysis of Thermo Scientific Pierce Chicken IgY purification. Chicken IgY was purified according to each manufacturer's instructions. The gel shows the analysis of 2µg of protein applied per well. The Pierce IgY Kit purified the chicken IgY to a purity level of > 85% using GelCode Blue Stain Reagent (Product # 24590). The competitor's product achieved only a 53% purity level. The arrow indicates intact IgY.

Affinity Purification of Specific Antibodies

Although Proteins A, G, A/G and L are excellent ligands for purification of total IgG from a sample, purification of an antibody specific for a particular antigen and free of contamination from other immunoglobulins is often required. This can be accomplished by immobilizing the particular antigen used for immunization so that only those antibodies that bind specifically to the antigen are purified in the procedure.

Successful affinity purification of antibody depends on effective presentation of the relevant epitopes on the antigen to binding sites of the antibody. If the antigen is small and immobilized directly to a solid support surface by multiple chemical bonds, important epitopes may be blocked or sterically hindered, prohibiting effective antibody binding. Therefore, it is best to immobilize antigens using a unique functional group (e.g., sulfhydryl on a single terminal cysteine in a peptide) and to use an activated support whose reactive groups occur on spacer arms that are several atoms long. For larger antigens, especially those with multiple sites of immobilization, the spacer arm length becomes less important since the antigen itself serves as an effective spacer between the support matrix and the epitope. Generally, if the antigen was crosslinked to a carrier protein to facilitate antibody production, best results are obtained when the antigen is immobilized for affinity purification using the same chemistry (e.g., reaction to primary amines, sulfhydryls, carboxylic acids or aldehydes). In this way, all epitopes will be available for antibody binding, allowing greater efficiency in purification and recovery of the specific immunoglobulin.

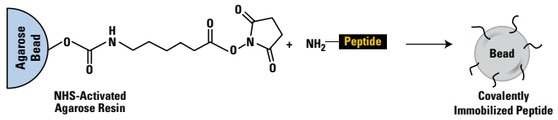
Little variation exists among typical binding and elution conditions for affinity purification of antibodies because at the core of each procedure is the affinity of an antibody for its respective antigen. Since antibodies are designed to recognize and bind antigens tightly under physiologic conditions, most affinity purification procedures use binding conditions that mimic physiologic pH and ionic strength. The most common binding buffers are phosphate-buffered saline (PBS) and Tris-buffered saline (TBS) at pH 7.2 and 1.5M NaCl. Once the antibody has been bound to an immobilized antigen, additional binding buffer is used to wash unbound material from the support. To minimize nonspecific binding, many researchers use wash buffer containing additional salt or detergent to disrupt any weak interactions.

Specific, purified antibodies are eluted from an affinity resin by altering the pH or ionic strength of the buffer. Antibodies generally are resilient proteins that tolerate a range of pH from 2.5 to 11.5 with minimal loss of activity, and pH-shift is by far the most common elution strategy. In some cases an antibody-antigen interaction is not efficiently disrupted by pH changes or is damaged by the pH, requiring that an alternate strategy be employed.

Immobilize Ligands through Primary Amines

NHS Ester Agarose

Thermo Scientific Pierce NHS-Activated Agarose Resin, available as a slurry or dry powder, allows for the simple and efficient immobilization of proteins to a beaded-agarose support. The activated agarose contains N-hydroxysuccinimide (NHS) ester with a spacer arm of at least 10 atoms in length that reacts with primary amines forming stable amide linkages. The NHS-Activated Agarose coupling reaction is performed in an amine-free buffer at pH 7-9, with typical coupling efficiencies of more than 85%. The prepared resin can be used for multiple affinity purification procedures. The crosslinked beaded agarose has fast linear flow potential, making it useful for gravity-flow and low- to medium-pressure applications.



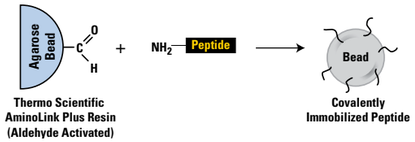
Target: –NH_2
Support: 6% agarose
Binding capacity: > 25mg protein/mL resin
Time: 30 minutes

Ordering Information

Product #	Description	Pkg. Size
26196	**Pierce NHS-Activated Agarose, Dry** Swell volume: 6-7.5mL/g of dry resin	1g
26197	**Pierce NHS-Activated Agarose, Dry** Swell volume: 6-7.5mL/g of dry resin	5g
26198	**Pierce NHS-Activated Agarose Spin Columns, 0.2mL** 25 spin columns containing 33mg of NHS-Activated Agarose Swell volume: 6-7.5µL/mg of dry resin	25 columns
26199	**Pierce NHS-Activated Agarose Spin Columns, 2mL** 5 spin columns containing 330mg of NHS-Activated Agarose Swell volume: 6-7.5µL/mg of dry resin	5 columns
26200	**Pierce NHS-Activated Agarose Slurry** 25mL of settled resin in anhydrous acetone	25mL

AminoLink Plus Resin

Thermo Scientific AminoLink® Plus Coupling Resin is crosslinked 4% beaded agarose that has been activated with aldehyde groups. Proteins and other molecules with primary amines can be covalently attached (immobilized) to AminoLink Resin to make chromatography columns for use in affinity purification. The aldehyde groups form stable secondary amine bonds with primary amines such as exist in the side chain of lysine (K) residues, which are generally abundant and readily accessible in proteins. AminoLink Plus is also on a highly crosslinked 4% agarose resin, making it more suitable for FPLC applications.



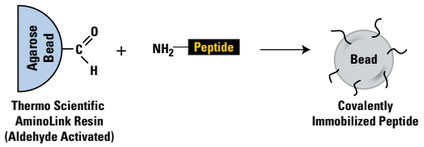
Target: –NH_2
Support: Highly crosslinked 4% agarose
Binding capacity: 20mg protein/mL resin
Time: 4 hours

Ordering Information

Product #	Description	Pkg. Size
44894	**AminoLink Plus Immobilization Kit** *Sufficient reagents for preparing five affinity columns.* Includes: AminoLink Column Phosphate Buffered Saline (PBS) Citrate-Carbonate Buffer Quenching Buffer Wash Solution Sodium Cyanoborohydride Solution (5 M) Column Accessories	Kit 5 x 2mL 1 pack 1 pack 50mL 240mL 0.5mL
20394	**AminoLink Plus Immobilization Trial Kit** *Sufficient reagents for preparing one affinity column.* Includes: AminoLink Column Phosphate Buffered Saline (PBS) Citrate-Carbonate Buffer Quenching Buffer Wash Solution Sodium Cyanoborohydride Solution (5 M) Column Accessories	Kit 1 x 2mL 1 pack 1 pack 15mL 50mL 0.5mL
20501	**AminoLink Plus Coupling Resin**	10mL
20505	**AminoLink Plus Coupling Resin**	50mL

AminoLink Coupling Resin

Thermo Scientific AminoLink Coupling Resin is crosslinked 4% beaded agarose that has been activated with aldehyde groups. Proteins and other molecules with primary amines can be covalently attached (immobilized) to AminoLink Resin to make chromatography columns for use in affinity purification. The aldehyde groups form stable secondary amine bonds with primary amines such as exist in the side chain of lysine (K) residues, which are generally abundant and readily accessible in proteins. Once a protein is immobilized, the prepared affinity resin can be used for a variety of batch and column affinity purification methods involving binding interactions with the immobilized protein. The resin and linkage are stable in most binding and elution conditions typically used in affinity chromatography, enabling prepared resin to be used for multiple rounds of affinity purification procedures.



Target: –NH_2
Support: 4% agarose
Binding capacity: 1-20mg protein/mL resin
Time: 4 hours

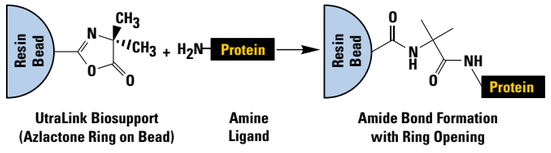
Ordering Information

Product #	Description	Pkg. Size
20381	**AminoLink Coupling Resin**	10mL
20382	**AminoLink Coupling Resin**	50mL
44890	**AminoLink Immobilization Kit** *Sufficient reagents to prepare five affinity columns.* *Each column can be used for up to 10 affinity purifications.* Includes: AminoLink Columns AminoLink Coupling Buffer Quenching Buffer Wash Solution, Sodium Cyanoborohydride Solution (5 M) Column Accessories	Kit 5 x 2mL 250mL 50mL 240mL 0.5mL
44892	**AminoLink Reductant** (Sodium cyanoborohydride)	2 x 1g

UltraLink Biosupport

Thermo Scientific UltraLink Biosupport is a durable polyacrylamide resin, activated to enable efficient and direct covalent immobilization of proteins and other biomolecules through their primary amines for use in affinity purification procedures.

UltraLink Biosupport is an alternative to CNBr- and NHS-activated agarose resins for immobilizing antibodies and other proteins to prepare affinity columns. The beaded resin is a hydrophilic copolymer of polyacrylamide and azlactone having a rigid polymeric structure with high surface area and pore volume. The azlactone groups react rapidly and efficiently with primary amines (e.g., side chain of lysine residues) to covalently immobilize protein or other amine-containing ligands. The bead structure and efficient coupling chemistry of UltraLink Biosupport results in high protein binding capacity, high linear flow rates, low nonspecific binding and overall superior performance in affinity chromatography. UltraLink Resins are ideal for medium pressure applications such as FPLC.



Target: –NH_2
Support: 4% agarose
Binding capacity: 1-20mg protein/mL resin
Time: 4 hours

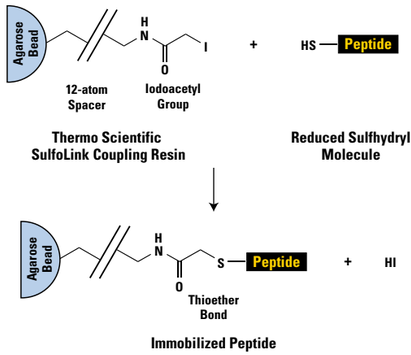
Ordering Information

Product #	Description	Pkg. Size
53110	UltraLink Biosupport (8-10mL)	1.25g
53111	UltraLink Biosupport (50mL)	6.25g

Immobilize Ligands through Reduced Sulfhydryls

SulfoLink Coupling Resin

Thermo Scientific SulfoLink® Coupling Resin is porous, crosslinked, 6% beaded agarose that has been activated with iodoacetyl groups. When incubated with a solution of peptide or protein that contains reduced cysteine residues, the iodoacetyl groups react specifically and efficiently with the exposed sulfhydryls (–SH) to form covalent and irreversible thioether bonds that permanently attach the peptide or protein to the resin. The result is a custom-made affinity resin for purification of antibodies, antigens and other molecules of interest.



Target: –SH
Support: 6% agarose or UltraLink® Resin
Binding capacity: 20mg protein/mL resin
Time: 2-3.5 hours

Ordering Information

Product #	Description	Pkg. Size	
44995	SulfoLink Immobilization Kit for Proteins *Sufficient reagents to prepare five affinity columns.* Kit contains:	Kit	
	SulfoLink Columns	5 x 2mL	
	SulfoLink Sample Preparation Buffer	7.5mL	
	SulfoLink Coupling Buffer	500mL	
	Wash Solution	120mL	
	2-Mercaptoethylamine•HCl	5 x 6mg	
	L-Cysteine•HCl	100mg	
	Zeba Desalt Spin Columns	5 x 5mL	
	Phosphate Buffered Saline	1 Pack	
	Columns Accessories		
44999	SulfoLink Immobilization Kit for Peptides *Sufficient reagents to prepare five affinity columns.* Kit contains:	Kit	
	SulfoLink Columns	5 x 2mL	
	SulfoLink Coupling Buffer	120mL	
	Wash Solution	120mL	
	Bond-Breaker TCEP Solution	0.5mL	
	L-Cysteine•HCl	100mg	
	Phosphate Buffered Saline	1 Pack	
	Column Accessories		
20325	SulfoLink Immobilization Trial Kit *Sufficient reagents to prepare 1 affinity column with a peptide or protein.* Kit contains:	Kit	
	SulfoLink Column	1 x 2mL	
	SulfoLink Sample Preparation Buffer	7.5mL	
	SulfoLink Coupling Buffer	120mL	
	Wash Solution	25mL	
	2-Mercaptoethylamine•HCl	6mg	
	Bond-Breaker TCEP Solution	0.5mL	
	Cysteine•HCl	100mg	
	Zeba Desalt Spin Column	5mL	
	Phosphate Buffered Saline	1 Pack	
	Columns Accessories		
20401	SulfoLink Coupling Resin	10mL	
20402	SulfoLink Coupling Resin	50mL	
20404	SulfoLink Coupling Resin	250mL	

Micro Peptide Coupling Kit

The Thermo Scientific Micro Peptide Coupling Kit enables covalent immobilization of cysteine-containing peptides for use in antibody purification and other affinity protocols. The Micro Peptide Kit is for immobilizing small amounts (25 to 250µg) of sulfhydryl-containing peptides (e.g., cysteine-terminated peptides) onto a beaded porous resin to create a small, reusable, microcentrifuge affinity column. The coupling and affinity purification procedures are optimized for small sample volumes (200 to 300µL). Wash and elution steps are achieved rapidly and efficiently with the convenient microcentrifuge spin columns. Each kit contains sufficient reagents for 10 coupling reactions and 20 affinity purifications. The kit is ideal for immobilizing peptide antigens that contain a terminal cysteine residue for use in purifying specific antibodies from small serum, ascites or culture supernatant samples.

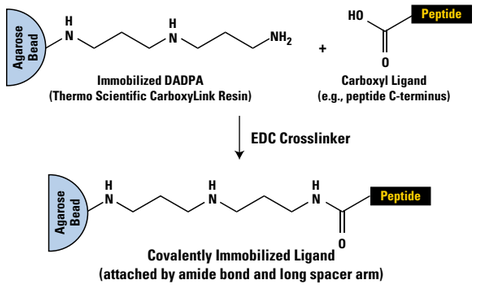
Ordering Information

Product #	Description	Pkg. Size	
20485	Micro Peptide Coupling Kit *Sufficient for coupling 10 sulfhydryl-containing peptides or proteins and perform 20 affinity purifications.* Kit contents:	Kit	
	UltraLink Iodoacetyl Spin Columns, 0.1mL	10 columns	
	Coupling Buffer	100mL	
	L-Cysteine-HCl	100mg	
	Wash Solution	25mL	
	PBS Pack (makes 500mL)	1 pack	
	IgG Elution Buffer	50mL	
	Collection Tubes	200 tubes	

Immobilize Ligands through Carboxylic Acids

CarboxyLink Coupling Resin

Thermo Scientific CarboxyLink® Coupling Resin and Kits provide for covalent immobilization of peptides or other carboxyl-containing (–COOH) molecules to a porous, beaded resin for use in affinity purification procedures. CarboxyLink Resin is crosslinked beaded agarose (or polyacrylamide UltraLink Support) that has been activated with diamino-dipropylamine (DADPA) to contain long spacer arms, each with a primary amine at the end. When incubated with the resin and the carbodiimide crosslinker EDC (included in the CarboxyLink Immobilization Kit), carboxyl-containing molecules become permanently attached to the support by stable amide bonds.



Target: –COOH
Support: 4% agarose or UltraLink® Resin
Binding capacity: 5mg protein/mL resin
Time: 4 hours

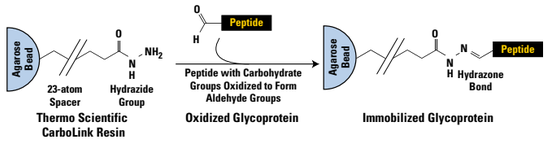
Ordering Information

Product #	Description	Pkg. Size
20266	CarboxyLink Coupling Resin	25mL
44899	CarboxyLink Immobilization Kit	Kit
	Kit contains: CarboxyLink Columns (DADPA agarose)	5 x 2mL
	EDC Crosslinker	5 x 60mg
	Coupling Buffer (MES-buffered Saline)	500mL
	Wash Solution (1 M NaCl)	120mL
	Column Accessories	
53154	CarboxyLink Immobilization Kit with UltraLink Support	Kit
	Kit contains: DADPA UltraLink Columns	5 x 2mL
	EDC Crosslinker	5 x 60mg
	Coupling Buffer (MES-buffered Saline)	500mL
	Wash Solution (1 M NaCl)	120mL
	Column Accessories	

Immobilize Ligands through Carbohydrates

CarboLink Coupling Resin

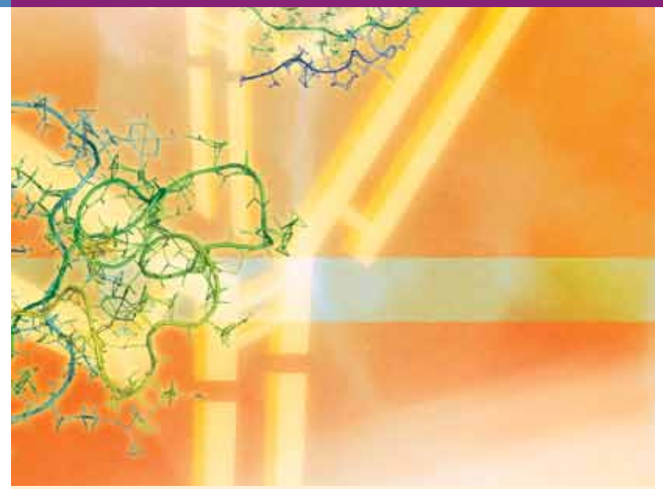
Thermo Scientific CarboLink® Coupling Resin and Kits provide for covalent immobilization of glycoproteins and other carbohydrate-containing molecules to beaded agarose (or polyacrylamide UltraLink Support) for use in affinity purification procedures. Carbohydrate moieties in glycoproteins contain common sugars whose cis-diol groups are easily oxidized with sodium meta-periodate (included in the CarboLink Kit) to yield aldehydes. When incubated with the CarboLink Resin, these aldehyde groups react spontaneously with the hydrazide group of the activated resin to form stable, covalent bonds.



Target: –CHO
Support: 6% agarose
Binding capacity: 5mg protein/mL resin
Time: 8 hours

Ordering Information

Product #	Description	Pkg. Size
20355	CarboLink Immobilization Trial Kit	Kit
	Kit contains: CarboLink Column	1 x 2mL
	CarboLink Coupling Buffer	60mL
	CarboLink Wash Solution	15mL
	Sodium meta periodate	1 x 5mg
	Zeba Desalting Column	1 x 5mL
44910	CarboLink Immobilization Kit	Kit
	Kit contains: CarboLink Columns	5 x 2mL
	CarboLink Coupling Buffer	250mL
	CarboLink Wash Solution	100mL
	Sodium meta periodate	5 x 5mg
	Zeba Desalting Columns	5 x 5mL
20391	CarboLink Coupling Resin	10mL
53149	UltraLink Hydrazide Resin	10mL



Antibody Fragmentation Overview

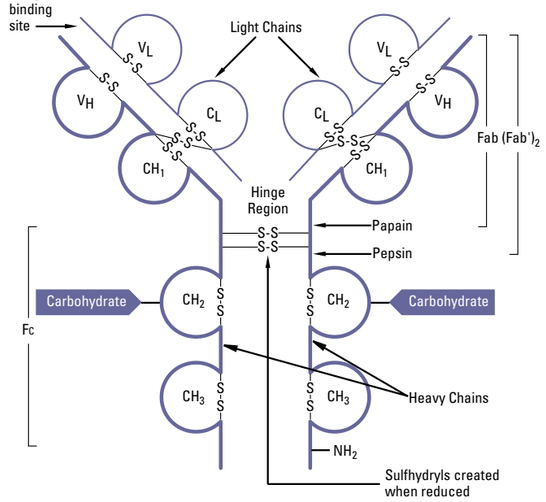
Often it is useful to study or make use of the activity of one portion of an immunoglobulin without interference from other portions of the molecule. It is possible to selectively cleave the immunoglobulin molecule into fragments that have discrete characteristics. Antibody fragmentation is accomplished using proteases that digest or cleave certain portions of the immunoglobulin protein structure. Although fragmentation of all immunoglobulin classes is possible, only procedures for fragmentation of mouse, rabbit and human IgG and IgM have been well-characterized. The two groups of antibody fragments of primary interest are antigen-binding fragments such as Fab and nonantigen-binding, class-defining fragments such as Fc. More than one type of antigen-binding fragment is possible, but each contains at least the variable regions of both heavy and light immunoglobulin chains (V_H and V_L, respectively) held together (usually by disulfide bridges) so as to preserve the antibody-binding site. Fc fragments consist of the heavy chain constant region (Fc region) of an immunoglobulin and mediate cellular effector functions.

Antibody fragmentation is somewhat laborious, requires optimization of enzyme-mediated digestion of the protein and necessitates an ample supply (e.g., 10mg) of antibody to make it reasonably efficient. For these reasons, fragmentation is usually performed only when the antibody of interest is available in large quantity and the particular application demands it.

Advantages of Antibody Fragments

Antibody fragments offer several advantages over intact antibody as reagents in an immunochemical technique:

- Using antigen-binding regions that have been separated from the Fc region reduces nonspecific binding that results from Fc interactions (many cells have receptors for binding to the Fc portion of antibodies).
- Small antigen-binding fragments generally provide higher sensitivity in antigen detection for solid-phase applications as a result of reduced steric hindrance from large protein epitopes.
- Because they are smaller, antibody fragments more readily penetrate tissue sections, resulting in improved staining for immunohistochemical applications.
- Antibody fragments are the best choice for antigen-antibody binding studies in the absence of Fc-associated effector functions (e.g., complement fixation, cell membrane receptor interaction).
- Antibody fragments offer a simple system by which to study the structural basis for immune recognition using X-ray crystallography or nuclear magnetic resonance.
- Antibody fragments have lower immunogenicity than intact antibody.



Antibody Fragmentation

Types of Antibody Fragments

$F(ab')_2$, Fab, Fab´ and Fv are antigen-binding fragments that can be generated from the variable region of IgG and IgM. These antigen-binding fragments vary in size (MW), valency and Fc content. Fc fragments are generated entirely from the heavy chain constant region of an immunoglobulin. The structures of these antibody fragments are illustrated in schematic form in Figure 1 and summarized below. In addition, several unique fragment structures can be generated from pentameric IgMs, including an "IgG"-type fragment, an inverted "IgG"-type fragment and a pentameric Fc fragment. IgM fragmentation is discussed in detail on pages 52-53.

$F(ab')_2$

$F(ab')_2$ (110,000 dalton IgG fragment, 150,000 dalton IgM fragment) fragments contain two antigen-binding regions joined at the hinge through disulfides. This fragment is void of most, but not all, of the Fc region.

Fab´

Fab´ (55,000 dalton IgG, 75,000 dalton IgM) fragments can be formed by the reduction of $F(ab')_2$ fragments. The Fab´ fragment contains a free sulfhydryl group that may be alkylated or utilized in conjugation with an enzyme, toxin or other protein of interest. Fab´ is derived from $F(ab')_2$; therefore, it may contain a small portion of Fc.

Fab

Fab (50,000 daltons) is a monovalent fragment that is produced from IgG and IgM, consisting of the V_H, C_{H1} and V_L, C_L regions, linked by an intrachain disulfide bond.

Fv

Fv (25,000 daltons) is the smallest fragment produced from IgG and IgM that contains a complete antigen-binding site. Fv fragments have the same binding properties and similar three-dimensional binding characteristics as Fab. The V_H and V_L chains of the Fv fragments are held together by noncovalent interactions. These chains tend to dissociate upon dilution, so methods have been developed to cross-link the chains through glutaraldehyde, intermolecular disulfides or a peptide linker.

"r IgG"

"r IgG" (80,000 daltons) is a reduced form of IgG composed of one complete light chain and one complete heavy chain. It is essentially one-half of an intact IgG molecule and it contains a single antigen-binding site. "r IgG" fragments are formed by the selective reduction of disulfide bonds in the hinge region of an antibody.

Fc

Fc (50,000 daltons) fragments contain the C_{H2} and C_{H3} region and part of the hinge region held together by one or more disulfides and noncovalent interactions (Figure 1). Fc and Fc5µ fragments are produced from fragmentation of IgG and IgM, respectively. The term Fc is derived from the ability of these antibody fragments to crystallize. Fc fragments are generated entirely from the heavy-chain constant region of an immunoglobulin. The Fc fragment cannot bind antigen, but it is responsible for the effector functions of antibodies, such as complement fixation.

$F(ab')_2$, Fab´, Fab and Fv fragments produced from IgM function in much the same way as $F(ab')_2$, Fab´, Fab and Fv fragments from IgG. However, compared to those in IgG, individual antigen-binding sites in IgM generally have lower binding affinities, which are compensated in the complete IgM by its pentameric form. The increased binding valency of $F(ab')_2$ may make it preferable to Fv and Fab fragments.

$F(ab')_2$ fragments are divalent, and they may be a superior alternative to Fab fragments for antibodies with low affinity. The $F(ab')_2$ fragments have higher avidity than the Fab and Fab´ fragments. $F(ab')_2$ fragments can precipitate antigen. Fab and Fab´ are univalent molecules that cannot precipitate antigen. Fab and Fab´ fragments have a decreased binding strength, and normally stable antigen-antibody complexes may dissociate during washes in certain applications.

Fragmentation of IgG

The hinge region of an immunoglobulin monomer (IgG) is readily accessible to proteolytic attack by enzymes. Cleavage at this point produces $F(ab')_2$ or Fab fragments and the Fc fragment. The Fc fragment may remain intact or become further degraded, depending upon the enzyme and conditions used. Proteolytic IgG fragmentation using three different enzymes is discussed below and summarized in Figure 2. Traditionally, IgG proteolysis was accomplished in solution using free enzyme. We have developed immobilized enzyme products that enable better control of the digestion and separation of reaction products from the protease.

Immobilized Papain

Papain is a nonspecific, thiol-endopeptidase that has a sulfhydryl group in the active site, which must be in the reduced form for activity. When IgG molecules are incubated with papain in the presence of a reducing agent, one or more peptide bonds in the hinge region are split, producing three fragments of similar size: two Fab fragments and one Fc fragment.[1] When Fc fragments are of interest, papain is the enzyme of choice because it yields a 50,000 dalton Fc fragment.

Papain is primarily used to generate Fab fragments, but it also can be used to generate $F(ab')_2$ fragments.[2] To prepare $F(ab')_2$ fragments, the papain is first activated with 10mM cysteine. The excess cysteine is then removed by gel filtration. If no cysteine is present during papain digestion, $F(ab')_2$ fragments can be generated. These fragments are often inconsistent, and reproducibility can be a problem. If the cysteine is not completely removed, overdigestion can be a problem.[2]

Figure 1. Structure of Fab, Fab´, $F(ab')_2$ and Fv fragments.

Crystalline papain is often used for the digestion of IgG; however, it is prone to autodigestion. Mercuripapain, which is less prone to autodigestion than crystalline papain, can be used; however, both of these non-immobilized enzymes require an oxidant to terminate digestion. Immobilized papain is the preferred reagent because it allows for easy control of the digestion reaction, as well as separation of enzyme from the crude digest. There is no need to develop an ion exchange method for separating the fragments from the enzyme. The use of immobilized papain will also prevent formation of antibody-enzyme adducts, which can occur when using the soluble form of sulfhydryl proteases (such as papain). These adducts can be detrimental to fragments in the presence of reductants.

Immobilization also increases stability of the enzyme against heat denaturation and autolysis and results in longer maintenance of activity. Regeneration of the papain is often possible after immobilization, resulting in decreased costs. Cleavage can be regulated by digestion time or flow rate through a column, yielding reproducible digests. Immobilized Papain (Product # 20341) offers all the advantages of immobilized enzyme supports (Figure 3). Thermo Scientific Pierce Fab Preparation Kit (Product # 44985) has been optimized for rabbit, human and mouse IgG digestions. Suggestions on how to vary the protocols for other species' IgG are provided with the kit.

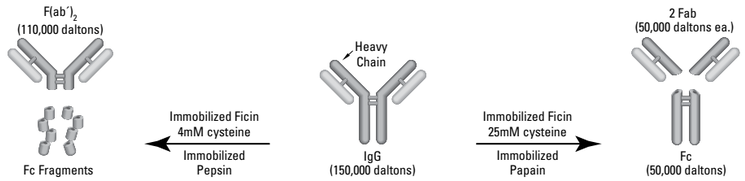


Figure 2. Use of papain, pepsin and ficin for IgG fragmentation.

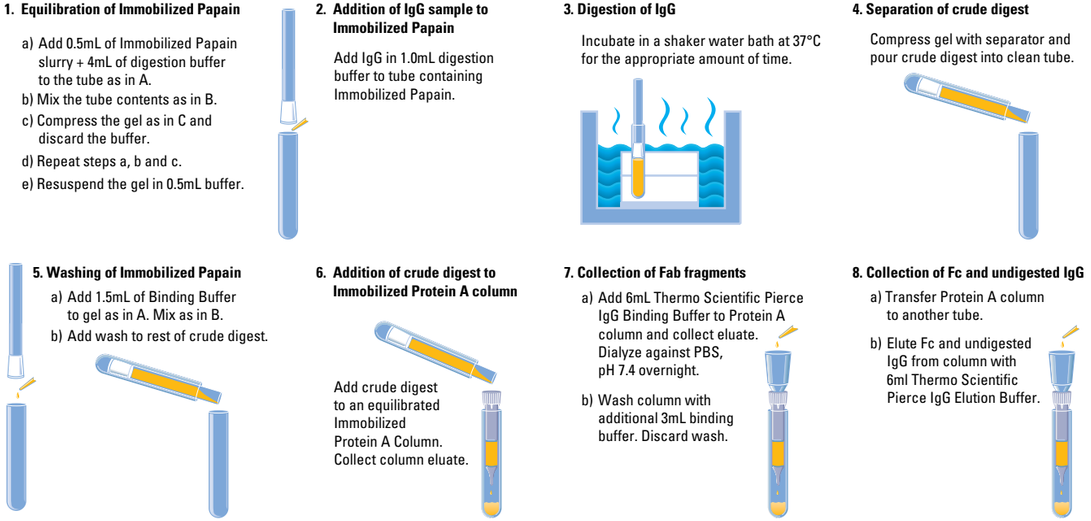


Figure 3. Preparation and isolation of Fab and Fc fragments with Thermo Scientific Pierce Immobilized Papain.

Immobilized Pepsin

Pepsin is a nonspecific endopeptidase that is active only at acid pH. It is irreversibly denatured at neutral or alkaline pH. Digestion by the enzyme pepsin normally produces one $F(ab')_2$ fragment and numerous small peptides of the Fc portion (Figure 4). The resulting $F(ab')_2$ fragment is composed of two disulfide-connected Fab units. The Fc fragment is extensively degraded, and its small fragments can be separated from $F(ab')_2$ by dialysis, gel filtration or ion exchange chromatography.

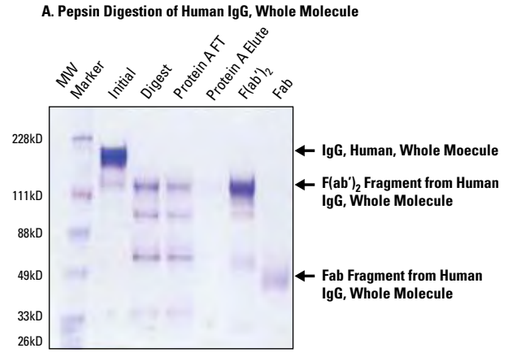


Figure 4. More efficient antibody digestion using Thermo Scientific Pierce Antibody Fragmentation Kits.

$F(ab')_2$ can be separated by mild reduction into two sulfhydryl-containing, univalent Fab´ fragments. The advantage of Fab´ fragments is that they can be conjugated to detectable labels directly through their sulfhydryl groups, ensuring that the active binding site remain unhindered and active. We offer 2-Mercaptoethylamine•HCl (2-MEA, Product # 20408) for mild reduction of $F(ab')_2$ fragments. For alternative labeling protocols, the free sulfhydryl may be blocked with an alkylating reagent, such as *N*-Ethylmaleimide (NEM, Product # 23030).

Immobilized Pepsin (Product # 20343) can be substituted for free pepsin in any application. Immobilized pepsin is advantageous because of its ability to immediately stop the digestion process, yielding reproducible digests. Immobilization of the enzyme allows for easy separation of the enzyme from the crude digest, eliminating the need to develop an ion exchange method for separating the fragments from the enzyme. Also, immobilization increases the stability of the pepsin against heat denaturation and autolysis, resulting in longer maintenance of activity. Thermo Scientific Pierce $F(ab')_2$ Preparation Kit (Product # 44988) has been optimized for human, rabbit and mouse IgG digestions. Suggestions on how to vary protocols for other species' IgG (IgG must bind to Protein A) are provided.

Immobilized Ficin

Ficin is a thiol protease that can digest mouse monoclonal IgG_1 into either $F(ab')_2$ or Fab fragments, depending on the concentration of cysteine used. Ficin will generate $F(ab')_2$ in the presence of 4mM cysteine. Fab fragments will be generated with ficin in the presence of 25mM cysteine (Figure 2).

Ficin cleavage produces $F(ab')_2$ fragments of nearly identical size to those obtained from IgG by pepsin but with immunoreactivities and affinities comparable to those of intact IgG_1 antibody.[3] By increasing the concentration of cysteine activator, Fab antigen-binding fragments can be generated.[4] The integrity of the resultant antigen-binding fragments is aided by the neutral pH conditions of the ficin digestion. The difficulties of using pepsin in this application makes ficin digestion the preferred method for producing $F(ab')_2$ fragments from murine monoclonal IgG_1. Although $F(ab')_2$ fragments have been generated from an IgG_1 antibody using preactivated papain,[5] stable, consistent product by papain is often difficult to obtain.[6]

Immobilized Ficin (Product # 44881) enables better control of the digestion reaction than free ficin, resulting in antibody fragments that are free of autodigestion products. In addition, the use of Immobilized Ficin eliminates the incorporation of ficin into antibody fragments. The Thermo Scientific Pierce IgG_1 Fab and $F(ab')_2$ Preparation Kit (Product # 44980) was developed to allow gentle production and purification of both Fab and $F(ab')_2$ fragments from intact murine IgG_1 antibodies. Immobilized Ficin can be used repeatedly to cleave an IgG_1 subclass antibody, yielding either Fab or $F(ab')_2$ fragments. The type of fragment produced is controlled by the specific concentration of cysteine activator used during the digestion.

References
1. Coulter, A. and Harris, R. (1983). *J. Immunol. Methods* **59**, 199-203.
2. Goding, J. (1983). *Monoclonal Antibodies: Principles and Practice.* Academic Press Inc., London, U.K.
3. Mariani, M., *et al.* (1991). *Mol. Immunol.* **28**, 69-77.
4. Sykaluk, L. (1992). Pierce Chemical Company, unpublished results.
5. Buguslawski, S.J., *et al.* (1989). *J. Immunol. Methods* **120**, 51-56.
6. Milenic, D.E., *et al.* (1989). *J. Immunol. Methods* **120**, 71-83.

Enzymes used for antibody digestion.

Enzyme	Molecular Weight	Ext. Coefficient A_{280} of 1%	Type	Specificity	pH Optimum	Activators (Enhancers)	Inhibitors	Imm. Enzyme (mg/mL BCA)	pI
Pepsin	35,000	14.7	Acid	Broad – prefer Phe, Met, Leu, Trp bonds	1 (1-5)		pH >6, epoxides	1	11
Papain	23,000	25	Thiol	Broad – prefer Arg, Lys, His, Gly, Tyr bonds	6.5 (4-9.5)	cysteine, sulfide, sulfite, cyanide, (EDTA) (NBS*) (acridine dye)	heavy metals, carbonyls, *N*-ethyl maleimide (NEM), *p*-chloromercuro-benzoate	9.6	1.5
Ficin	26,000	21	Thiol	Uncharged or aromatic amino acids	6.5 (4-9.5)	cysteine, sulfide, sulfite, cyanide, (EDTA) (NBS) (acridine dye)	heavy metals, carbonyls, NEM, *p*-chloromercuro-benzoate		1.5
Trypsin	24,000	14.3	Serine	Arg, Lys	8	Ca^{2+} acts as a stabilizer	organophosphorous compounds, DFP**, benzimidine	10.5	1.5

**NBS = N-bromosuccinimide **DFP = diisopropyl fluorophosphate*

Fab Preparation Kit

The easiest, most convenient way to generate Fab fragments from IgG.

The Thermo Scientific Piece Fab Preparation Kit contains the reagents to digest human, rabbit or mouse IgG molecules into Fab fragments and Fc fragments by using immobilized papain. After digestion, the fragments are purified on an immobilized Protein A column provided in the kit. Detailed instructions allow for flexibility in the protocol for hard-to-digest antibodies.

Two formats of Fab Preparation Kits are available depending on the IgG sample size. Our standard Fab Preparation Kit enables Fab generation from 0.25 to 4mg IgG in a single use. The Pierce Fab Micro Preparation Kit is designed for Fab generation from 50 to 250µg, in a single use.

Highlights of Fab Fragments:

- Will not be affected by Fc receptors on cells such as macrophages, B cells, T cells, neutrophils and mast cells
- Will not precipitate antigen
- Easier to make and purify than $F(ab')_2$ fragments
- More rapid clearance of radiolabeled fragments from normal tissue than whole IgG conjugates
- Reduced immunogenicity (as a result of Fc region absence), minimizes human anti-mouse immunoglobulin (HAMA) response
- Fragments are less susceptible to phagocytosis

Antibody Fragment Applications:

- Immunohistochemistry
- Immunoassays, including *in vitro* diagnostic assays
- Radioimmunolocalization for tumor detection and radiotherapy
- Immunotargeting through the use of fragment conjugates as immunotoxins
- Crystallographic study of antibody-binding sites
- Study of Fc-binding proteins and effector functions

Highlights of Fc Fragments:

- Useful for studying effector functions of IgG without interference from antigen-binding sites
- Fc fragments can be used as blocking agents for histochemical staining

References
Coulter, A. and Harris, R. (1983). *J. Immunol. Methods* **59**, 199-203.
Drewett, J.G., *et al.* (1995). *J. Biol. Chem.* **270**, 4668-4674.
Mage, M.G. (1980). *Methods Enzymol.* **70**, 142-150.
Milenic, D.E., *et al.* (1989). *J. Immunol. Methods* **120**, 71-83.
Rousseaux, J., *et al.* (1983). *J. Immunol. Methods* **64**, 141-146.
Schmalzing, D., *et al.* (1995). *Anal. Chem.* **67**(3), 606-612.
Wolf, A., *et al.* (1994). *J. Biol. Chem.* **269**, 23051-23058.

Ordering Information

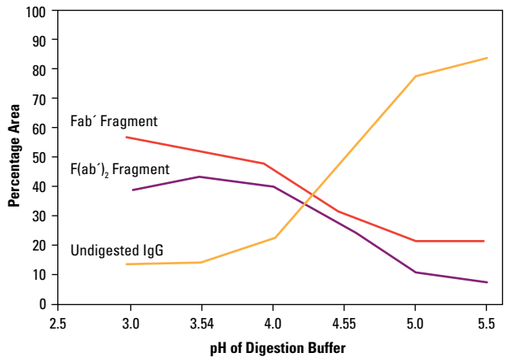
Product #	Description	Pkg. Size
44985	**Fab Preparation Kit** *Isolates and purifies Fab fragments from up to 10 antibody samples containing 0.25-4mg IgG.* Includes: Immobilized Papain Cysteine•HCl Fab Digestion Buffer NAb Protein A Spin Column Phosphate Buffered Saline IgG Elution Buffer Zeba Desalt Spin Columns Spin Columns and Accessories	Kit 1.25mL 1g 120mL 1mL 2 packs 120mL 10 x 2mL
44685	**Pierce Fab Micro Preparation Kit** *Sufficient for 10 x 250µg antibody fragmentation reactions.* Includes: Immobilized Papain Fab Digestion Buffer Cysteine•HCl Fab Digestion Buffer NAb Protein A Spin Column Phosphate Buffered Saline IgG Elution Buffer Zeba Desalt Spin Columns Spin Columns and Accessories	Kit 0.5mL 1g 1g 55mL 2 x 0.2mL 2 packs 50mL 10 x 0.5mL
20341	**Immobilized Papain**	5mL

F(ab´)$_2$ Preparation Kit

The easiest, most convenient way to generate F(ab´)$_2$ fragments from IgG.

Thermo Scientific Pierce F(ab´)$_2$ Preparation Kit contains reagents for digesting antibodies into F(ab´)$_2$ fragments that retain antigen-binding activity. Using immobilized pepsin allows the antibody digest to be free of any enzyme contaminants. Purifying F(ab´)$_2$ fragments is as easy as passing the solution over an immobilized protein A column. Detailed instructions for F(ab')$_2$ purification from human, rabbit or mouse are included. Allow for flexibility in the protocol for hard-to-digest antibodies.

Two formats of the F(ab')$_2$ Preparation Kit are available depending on the IgG sample size. Our standard F(ab')$_2$ Preparation Kit enables F(ab')$_2$ generation from 0.25 to 4mg IgG in a single use. Pierce F(ab')$_2$ Micro Preparation Kit is designed from F(ab')$_2$ generation from 50 to 250mg in a single use.



High-performance liquid chromatographic studies indicate the optimal pH for generation of F(ab´)$_2$ fragments from immobilized pepsin for human IgG.

Highlights:

- Will not be affected by Fc receptors on cells such as macrophages, B cells, T cells, neutrophils and mast cells
- They are divalent, which is recommended for retaining antigen-binding capabilities of low affinity antibodies
- Will precipitate antigent

References
Kulkarni, P.N., *et al.* (1985). *Cancer Immunol. Immunother.* **19**, 211-214.
Lamoyi, E. (1986). *Methods Enzymol.* **121**, 652-663.
Pruimboom, I.M., *et al.* (1999). *Infect. Immun.* **67(3)**, 1292-1296.
Rousseaux, J., *et al.* (1983). *J. Immunol. Methods* **64**, 141-146.
Wedrychowski, A., *et al.* (1993). *Biotechnology (N-Y)* **11**, 486-489.
Smith-Jones, P.M., *et al.* (2004). *Nature Biotech.* **22**, 32-37.

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
44688	**Pierce F(ab´)$_2$ Micro Preparation Kit** *Sufficient for 10 x 250µg antibody fragmentation reactions.* Includes: Immobilized Pepsin F(ab')$_2$ Digestion Buffer NAb Protein A Spin Column IgG Elution Buffer BupH PBS Packs Zeba Desalt Spin Columns Microcentrifuge Tubes Column Accessories	Kit 0.5mL 55mL 2 x 0.2mL 50mL 2 ea. 10 x 0.5mL 30 ea.	$335
44988	**Pierce F(ab´)$_2$ Preparation Kit** *Sufficient for 10 x 4mg antibody fragmentation reactions.* Includes: Immobilized Pepsin F(ab')$_2$ Digestion Buffer NAb Protein A Spin Column IgG Elution Buffer BupH PBS Packs Zeba Desalt Spin Columns Microcentrifuge Tubes Column Accessories	Kit 1.25mL 120mL 1 x 1mL 120mL 2 ea. 10 x 2mL 30 ea.	$450
20343	**Immobilized Pepsin** Support: Crosslinked 6% beaded agarose Activity: > 2,000 units per mL of settled gel Loading: 2-3mg/mL of gel	5mL	$160

IgG$_1$ Fab and F(ab´)$_2$ Preparation Kit

Generate both Fab and F(ab´)$_2$ fragments from monoclonal IgG$_1$ antibodies.

Problems with digesting mouse monoclonal IgG$_1$ can now be overcome by using immobilized Ficin. Ficin cleavage produces F(ab´)$_2$ fragments of nearly identical size to those obtained from IgG by pepsin, but with immunoreactivities and affinities comparable to those of the intact IgG$_1$ antibody. Similarly, by increasing the concentration of cysteine activator in the digestion buffer, Fab fragments can be created from the original IgG.

Two formats of the F(ab) and F(ab')$_2$ Preparation Kit are available depending on the IgG sample size. Our standard F(ab')$_2$ Preparation Kit enables F(ab) and F(ab')$_2$ generation from 0.25 to 4mg IgG in a single use. Pierce F(ab) and F(ab')$_2$ Micro Preparation Kit is designed from F(ab) and F(ab')$_2$ generation from 50 to 250mg in a single use.

Highlights:

- Can generate both Fab and F(ab´)$_2$ fragments from mouse IgG$_1$
- Reaction can be easily controlled
- Antibody fragments are free of autodigestion products
- Ficin contamination into antibody fragments is eliminated

References
Kurkela, R., *et al.* (1988). *J. Immunol.* **110**, 229-236.
Mariani, M., *et al.* (1991). *Mol. Immunol.* **28**, 69-77.
Sykaluk, L. (1992). Unpublished company results.
Wilson, K.M., *et al.* (1991). *J. Immunol. Methods* **138**, 111-119.

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
44680	**Pierce Mouse IgG Fab and F(ab´)$_2$ Micro Preparation Kit** *Sufficient for 10 x 250µg antibody fragmentation reactions.* Includes: Immobilized Ficin IgG1 Digestion Buffer Cysteine•HCl NAb Protein A Spin Column Protein A Binding Buffer IgG Elution Buffer BupH PBS Packs Zeba Desalt Spin Columns Microcentrifuge Tubes Column Accessories	Kit 0.8mL 120mL 1g 2 x 0.2mL 50mL 50mL 2 ea. 10 x 0.5mL 30 ea.	$387
44980	**Pierce Mouse IgG Fab and F(ab´)$_2$ Micro Preparation Kit** *Sufficient for 10 x 4mL antibody fragmentation reactions.* Includes: Immobilized Ficin IgG1 Digestion Buffer Cysteine•HCl NAb Protein A Spin Column IgG Elution Buffer BupH PBS Packs Zeba Desalt Spin Columns Microcentrifuge Tubes	Kit 2.5mL 120mL 1g 1 x 1mL 120mL 2 ea. 10 x 2mL 30 ea.	$499
44881	**Immobilized Ficin**	5mL	$173
44889	**Cysteine•HCl**	5g	$ 44

Fragmentation of IgM

IgM is an extremely large molecule that has a tendency to interact with other molecules and matrices besides the antigen. The large size of IgM creates difficulties in applications in which IgM is used for *in vitro* experiments. Intact IgM does not effectively penetrate tissues for immunohistochemical studies; it is necessary to produce smaller, active fragments for these studies. Also, because IgM molecules have difficulty permeating cell membranes, they are not ideal for use *in vivo*. Fragments are cleared more rapidly than intact IgM.

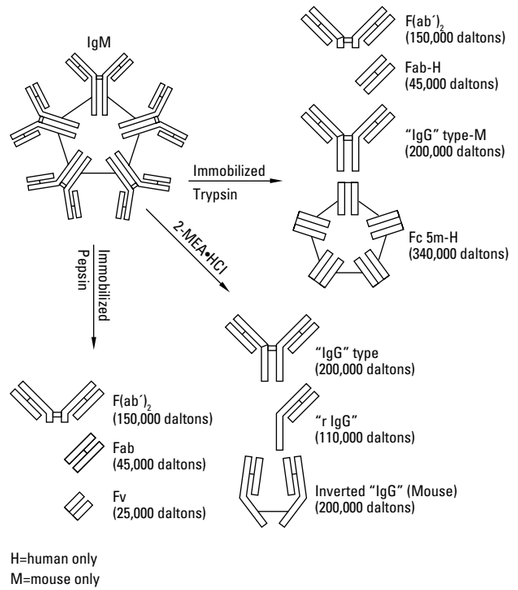
Each species of IgM reacts differently to enzymatic cleavage and reduction. For example, the relative structure of mouse and human IgM differ in the manner in which the monomers are linked to give the pentameric form, primarily as a result of differences in the location of disulfides between the monomers.[1] Oligosaccharide components, which may hinder enzymatic cleavage, also vary between species. Therefore, optimal digestion and reduction conditions for one species may prove ineffective for another.

Fragmentation of IgM by proteolyic enzymes proceeds differently from IgG fragmentation. These changes are related to differences in structure. The heavy (µ) chains are folded into multiple globular domains, and IgM has an actual domain (Cµ2) in place of a hinge region. Cµ2 lacks the proline-rich sequence that is found in the hinge region of IgG. This proline sequence makes the hinge more susceptible to cleavage. Also, IgM has a large carbohydrate portion in the Cµ2, which may sterically interfere with the action of proteolytic enzymes.

Enzymes and Reagents for IgM Fragmentation

Papain is a nonspecific, thiol-endopeptidase that has a sulfhydryl group in the active site, which must be in the reduced form for activity. Papain has been shown to produce heterogeneous fragments from IgM. Oligosaccharides in the hinge region of IgM interfere with papain digestion, causing a cleavage shift of 3-5 amino acids in either direction.

Pepsin is a nonspecific endopeptidase that is active only at an acid pH, and it is irreversibly denatured at neutral or alkaline pH. It is possible to produce F(ab´)$_2$, Fab and Fv fragments using pepsin to digest IgM (Figure 5). Many methods have been developed that use pepsin to produce different IgM fragments from different species.[2]



H=human only
M=mouse only

Figure 5. Fragmentation of IgM.

Trypsin is a serine protease that reacts optimally at pH 8.0. In general, increasing the enzyme/substrate ratio and/or temperature will increase the rate of digestion. Trypsin can generate F(ab´)$_2$, Fab, "IgG"-type and Fc5µ fragments from IgM (Figure 5). Trypsin digestion of several species of IgM was studied using trypsin with and without urea pretreatment.[2] Urea alters the susceptibility of the domains to digestion and produces different fragments than those digested in aqueous buffer. Many other procedures have been developed to digest IgM using trypsin.[3]

Mild reduction can be achieved using 2-Mercaptoethylamine•HCl (2-MEA, Product # 20408). Reduction will vary among IgM species, but an "IgG"-type and/or reduced IgG ("rIgG") should be formed in varied proportions, depending upon reduction time and/or temperature (Figure 5).[4] Fragmentation of mouse IgM also produces an inverted "IgG"-type fragment.

IgM Fragmentation Kit

The Thermo Scientific Pierce IgM Fragmentation Kit (Product # 44887) allows for the quick, easy production of fragments from mouse and human IgM. This kit can be used for species other than human and mouse; however, the protocols have not been optimized for all species. The kit contains everything needed to produce IgM fragments using trypsin, pepsin and 2-MEA protocols. The trypsin and pepsin are supplied in immobilized forms, eliminating the need to separate enzyme from the IgM fragments. The IgM is digested as it passes through the prepacked immobilized enzyme columns. The enzyme remains bound to the support matrix, ensuring there is no autodigested enzyme to contaminate the IgM fragments. Also, the immobilized enzymes offer increased stability against heat denaturation and autolysis. Regeneration of the immobilized enzymes makes the process more cost-efficient. The cleavage of IgM is easily regulated by adjustment of incubation times. Sample concentrators are included in the kit for easy fragment separation and concentration. Complete instructions and protocols are also included.

References
1. Milstein, C.P., *et al.* (1975). *Biochem. J.* **151**, 615-624.
2. Beale, D. and Van Dort, T. (1982). *Comp. Biochem. Physiol.* **71B(3)**, 475-482.
3. Plaut, A.G. and Tomasi, Jr., T.B. (1970). *Proc. Natl. Acad. Sci. USA* **65(2)**, 318-322.
4. Bevan, M.J., *et al.* (1972). *Progr. Biophys. Molec. Biol.* **25**, 131.

IgM Fragmentation Kit

Makes IgM fragmentation easy!

The large size of IgM creates difficulties in applications in which IgM is used for *in vitro* experiments. Intact IgM does not effectively penetrate tissues for immunohistochemical studies, therefore it is necessary to produce smaller, active fragments for *in vitro* or *in vivo* studies. We created the Thermo Scientific Pierce IgM Fragmentation Kit with immobilized trypsin and pepsin to easily generate a variety of IgM fragments (Figure 5).

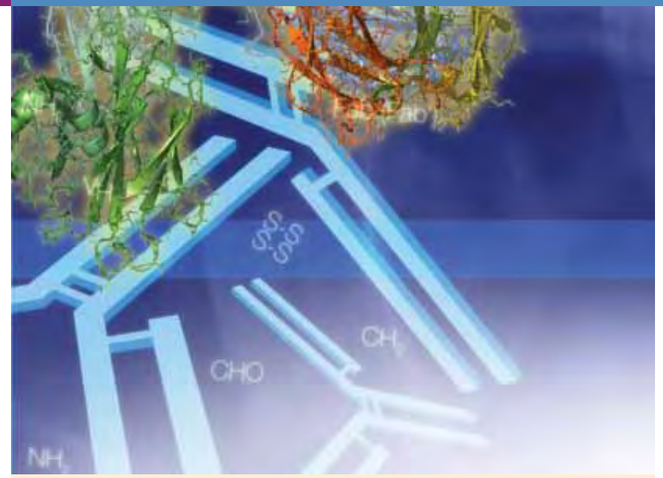
Highlights:

- Immobilized trypsin can generate F(ab´)$_2$, Fab, "IgG"-type and Fc(5µ) fragments from IgM
- Immobilized pepsin can produce F(ab´)$_2$, Fab and Fv fragments from IgM
- Complete kit, including detailed instructions, to digest and purify IgM fragments
- Immobilized enzymes prevent enzyme contaminants in final fragment preparation

References
Beale, D. and Van Dort, T. (1982). *Comp. Biochem. Physiol.* **[B] 71(3)**, 475-482.
Chen, F.-M. and Epstein, A.L. (1988). *Antibody, Immunoconjugates, and Radiopharmaceuticals* **1(4)**, 333-341.
Herremans, T., *et al.* (2000). *Clin. Diagn. Lab Immunol.* **7(1)**, 40-44.
Kakimoto, K. and Onoue, K. (1974). *J. Immunol.* **112(4)**, 1373-1382.
Lin, L.-C. and Putnam, F.W. (1978). *Proc. Natl. Acad. Sci. USA* **75(6)**, 2649-2653.
Poncet, P., *et al.* (1988). *Mol. Immunol.* **25(10)**, 981-989.

Ordering Information

Product #	Description	Pkg. Size
44887	**IgM Fragmentation Kit**	Kit
	Fragments up to 26mg of human or mouse IgM.	
	Includes: Immobilized Trypsin Columns	2 x 2mL
	Immobilized Pepsin Columns	2 x 2mL
	2-Mercaptoethylamine	6mg
	IgM Digestion Buffer	400mL
	IgM F(ab´)$_2$ Digestion Buffer	200mL
	Iodoacetamide	
	Sample Concentrators	
	Desalting Columns	2 x 5mL
20343	**Immobilized Pepsin**	5mL
20408	**2-Mercaptoethylamine•HCl**	6 x 6mg

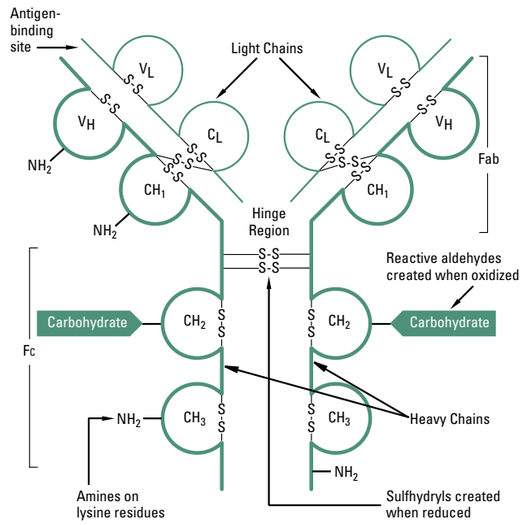


Antibody Structure and Modification Sites

Antibodies, like other proteins, can be covalently modified in many ways to suit the purpose of a particular assay. Many immunological methods involve the use of labeled antibodies and a variety of reagents have been created to allow labeling of antibodies. Enzymes, biotin, fluorophores and radioactive isotopes are all commonly used to provide a detection signal in biological assays.

Understanding the functional groups available on an antibody is the key to choosing the best method for modification, whether that be for labeling, crosslinking or covalent immobilization. Most antibody labeling strategies use one of three targets:

- **Primary amines (–NH$_2$):** these occur on lysine residues and the N-terminus of each polypeptide chain. They are numerous and distributed over the entire antibody.
- **Sulfhydryl groups (–SH):** these occur on cysteine residues and exist as disulfide bonds that stabilize the whole-molecule structure. Hinge-region disulfides can be selectively reduced to make free sulfhydryls available for targeted labeling.
- **Carbohydrates (sugars):** glycosylation occurs primarily in the Fc region of antibodies (IgG). Component sugars in these polysaccharide moieties that contain cis-diols can be oxidized to create active aldehydes (–CHO) for coupling.



Antibody structure and labeling sites.

Primary Amines as Antibody Labeling Sites

The most common target for antibody labeling or conjugation is primary amines, which are found primarily on lysine residues. They are abundant, widely distributed and easily modified because of their reactivity and their location on the surface of the antibody.

Primary amines can be targeted using several kinds of conjugation chemistries. The most specific and efficient reagents are those that use the N-hydroxysuccinimidyl ester (NHS ester) reactive group. Many biotinylation and fluorescent labeling products are commercially available pre-activated with NHS-ester group.

Other amine-targeted strategies are commonly used to conjugate antibodies to enzymes such as horseradish peroxidase (HRP) or alkaline phosphatase (AP); these include glutaraldehyde and reductive amination crosslinking approaches. Thermo Scientific AminoLink Plus Coupling Resin uses reductive amination to covalently immobilized antibodies through primary amines.

In any particular antibody clone, lysines (primary amines) might occur prominently within the antigen binding site. Thus, the lone drawback to this labeling strategy is that it occasionally causes a significant decrease in the antigen-binding activity of the antibody. The decrease may be particularly pronounced when working with monoclonal antibodies or when attempting to add a high density of labels per antibody molecule.

Sulfhydryls as Antibody Labeling Sites

The second useful target for covalently labeling antibodies is sulfhydryls. These groups exist in proteins under reducing conditions but more often are found in native proteins (including antibodies) in oxidized form as disulfide bonds (cystine). Disulfide bonds are important contributors to antibody function as they participate in the tertiary structure of each subunit, covalently connect heavy and light chains, and connect the two antibody halves at the hinge region.

Conjugation at sulfur atoms requires that the thiols exist as free suflhydryls. Thus, to label an antibody, at least some of the native disulfide bonds must be cleaved with reducing agents. Because disulfides in the hinge region are the most susceptible to reduction, it is possible to selectively cleave only these disulfides and thereby to split the antibody into monovalent halves without damaging the remaining structure and antigen-binding sites.

When it can be accomplished, labeling antibodies at hinge-region sulfhydryls ensures consistent labeling at a defined location. In contrast with amines-targeted labeling, this provides greater certainty that antigen binding sites will not be inactivated and that the population of antibody molecules in a sample will acquire the same density of label.

Reagents that are activated with maleimide or iodoacetyl groups are the most effective for sulfhydryl-directed conjugation. Many biotin, fluorescent and enzyme labeling reagents are available pre-activated with maleimide groups. Thermo Scientific SulfoLink Coupling Resin uses iodoacetyl chemistry to immobilize antibodies through sulfhydryl groups.

Carbohydrates as Antibody Labeling Sites

The third useful target for labeling antibodies is carbohydrate moieties. Because glycosylation sites in antibodies are predominantly found on the Fc portion of the antibody, they can often be modified without significantly affecting the antigen-binding capacity.

Labeling carbohydrates requires more steps than labeling amines because the carbohydrates must first be oxidized to create reactive aldehydes; however, the strategy generally results in antibody conjugates with high activity.

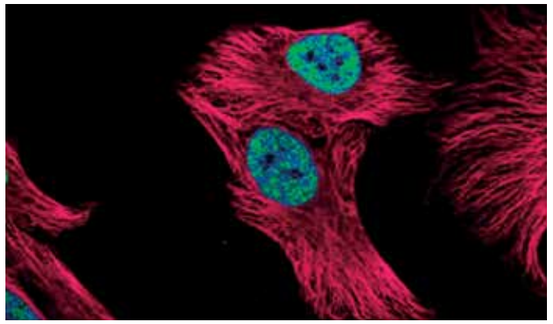
Aldehyde-activated (oxidized) sugars can be reacted directly to primary amines through reductive amination (mentioned above) or to reagents that have been activated with hydrazide groups. Several hydrazide-activated biotinylation reagents are available. Thermo Scientific CarboLink Coupling Resin uses reductive amination to immobilize antibodies through carbohydrate groups that have been oxidized.

Bright New Alternatives to Alexa Fluor®, CyDye® and LI-COR Fluorescent Dyes

Thermo Scientific DyLight Dyes have absorption spectra ranging from 353nm to 770nm (Table 1) and match the principal output wavelengths of common fluorescence instrumentation. The DyLight Dyes exhibit higher fluorescence intensity and photostability than Alexa Fluor, CyDye and LI-COR Dyes in many applications and remain highly fluorescent over a broad pH range (pH 4-9). Additionally, the water solubility of the DyLight Dyes allows a high dye-to-protein ratio without precipitation during conjugation.

Highlights:

- Available in both amine- and sulfhydryl-reactive chemistries for fast and efficient labeling of IgG or other proteins
- High water solubility
- Excellent photostability
- Compatible with common fluorescence instrumentation



Thermo Scientific DyLight 488 and DyLight 633 Dyes exhibit outstanding fluorescence in structured illumination. The uniform fluorescence intensity throughout the images demonstrates the outstanding brightness and photostability of DyLight 488 and 633 Dyes. **Red:** Alpha tubulin detected in HeLa cells with anti-tubulin monoclonal antibody and DyLight 633 Dye-conjugated secondary antibody (highly cross-adsorbed). **Green:** Histone H4 detected with anti-histone monoclonal antibody and DyLight 488 Dye-conjugated secondary antibody (highly cross-adsorbed). **Blue:** Nucleus counter-stained with fluorescent mounting media containing DAPI. Images were acquired with the Axio Imager Z1 and ApoTome™ Slider (Zeiss MicroImaging, Inc). The ApoTome Module provides confocal-like resolution allowing optical sectioning without using a pinhole (e.g., confocal). No image enhancement was performed.

Ordering Information

Product #	Description	Pkg. Size
Amine-Reactive Dyes		
46426	DyLight 350 NHS Ester	1mg
46427	DyLight 350 NHS Ester	5 x 65µg
46400	DyLight 405 NHS Ester	1mg
46401	DyLight 405 NHS Ester	5 x 50µg
46402	DyLight 488 NHS Ester	1mg
46403	DyLight 488 NHS Ester	5 x 50µg
46407	DyLight 549 NHS Ester	1mg
46408	DyLight 549 NHS Ester	5 x 50µg
46412	DyLight 594 NHS Ester	1mg
46413	DyLight 594 NHS Ester	5 x 65µg
46414	DyLight 633 NHS Ester	1mg
46417	DyLight 633 NHS Ester	5 x 50µg
46415	DyLight 649 NHS Ester	1mg
46416	DyLight 649 NHS Ester	5 x 50µg
46418	DyLight 680 NHS Ester	1mg
46419	DyLight 680 NHS Ester	5 x 50µg
53068	DyLight 680B NHS Ester	1mg
53069	DyLight 680B NHS Ester	5 x 50µg
46420	DyLight 750 NHS Ester	1mg
46423	DyLight 750 NHS Ester	5 x 50µg
46421	DyLight 800 NHS Ester	1mg
46422	DyLight 800 NHS Ester	5 x 50µg
Sulfhydryl-Reactive Dyes		
46600	DyLight 405 Maleimide	1mg
46602	DyLight 488 Maleimide	1mg
46607	DyLight 549 Maleimide	1mg
46608	DyLight 594 Maleimide	1mg
46613	DyLight 633 Maleimide	1mg
46615	DyLight 649 Maleimide	1mg
46618	DyLight 680 Maleimide	1mg
46620	DyLight 680B Maleimide	1mg
46619	DyLight 750 Maleimide	1mg
46621	DyLight 800 Maleimide	1mg

Label and Purify Antibodies in One Hour

Antibody labeling kits for fast and efficient labeling and exceptional recovery.

The Thermo Scientific DyLight Antibody Labeling Kits were specifically developed for fast, efficient labeling of antibodies. Two convenient kit formats are available to accommodate varied labeling requirements. The Antibody Labeling Kits contain all necessary components to perform three separate labeling reactions using 1mg of IgG or similar quantities of other proteins. The DyLight Microscale Antibody Labeling Kits contain all the necessary components to perform five separate labeling reactions using 100µg of IgG.

The labeling kits use high-performance spin desalting columns to provide exceptional dye removal and antibody recovery (Figure 1).

Highlights:

- Fast – fluorescently label and purify protein in approximately one hour
- Amine-reactive dyes – label virtually any protein
- Pre-measured fluorescent dye – eliminate the time, waste and hassle associated with weighing dye
- Efficient non-reacted dye removal
- Minimal sample dilution
- Spin column format eliminates the need for column preparation, fraction screening and waiting for protein to emerge from column
- Easy protocol

Figure 1. Thermo Scientific DyLight Antibody Labeling Kits provide outstanding recovery. The percent recovery for DyLight Antibody Labeling Kits is the average for 16 labeling reactions using three different antibodies. The percent recovery for DyLight Microscale Antibody Labeling Kits is the average for 15 labeling reactions using three different antibodies.

Spectral properties of Thermo Scientific DyLight Fluorescent Dyes.

Emission	DyLight Dye	Ex/Em*	ε†	Spectrally Similar Dyes
Blue	350	353/432	15,000	AMCA, Alexa Fluor 350 Dye
Blue	405	400/420	30,000	Alexa Fluor 405 and Cascade Blue Dyes
Green	488	493/518	70,000	Alexa Fluor 488, fluorescein and FITC Dyes
Yellow	549	560/574	150,000	Alexa Fluor 546, Alexa Fluor 555, Cy®3 and TRITC Dyes
Red	594	593/618	80,000	Alexa Fluor 594 and Texas Red® Dyes
Red	633	638/658	170,000	Alexa Fluor 633 Dye
Red	649	654/673	250,000	Alexa Fluor 647 and Cy5 Dyes
Near-IR	680	692/712	140,000	Alexa Fluor 680 and Cy5.5 Dyes
Near-IR	680B	679/702	185,000	Alexa Fluor 680, DyLight 680
Near-IR	750	752/778	220,000	Alexa Fluor 750 and Cy7 Dyes
Near-IR	800	777/790	270,000	IRDye® 800 Dye

**Excitation and emission maxima in nanometers (± 4nm)* *† Molar extinction coefficient ($M^{-1}cm^{-1}$)*

Microscale Kits

Contain sufficient reagents to label and purify 5 x 100µg of IgG.

In addition to contents listed below, all Microscale Kits include:

- Reaction Buffer, 1mL
- Spin Columns, 5 each
- Microcentrifuge Collection Tubes, 10 each
- Purification Resin, 5mL

Ordering Information

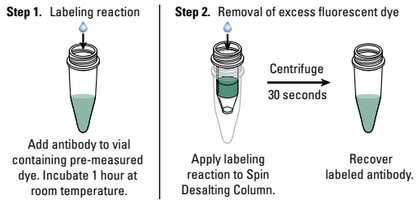
Product #	Description	Pkg. Size
62276	DyLight 350 Microscale Antibody Labeling Kit DyLight 350 NHS Ester, 5 vials	Kit 5 vials
53021	DyLight 405 Microscale Antibody Labeling Kit DyLight 405 NHS Ester	Kit 5 vials
53025	DyLight 488 Microscale Antibody Labeling Kit DyLight 488 NHS Ester	Kit 5 vials
53035	DyLight 549 Microscale Antibody Labeling Kit DyLight 549 NHS Ester	Kit 5 vials
53045	DyLight 594 Microscale Antibody Labeling Kit DyLight 594 NHS Ester	Kit 5 vials
53047	DyLight 633 Microscale Antibody Labeling Kit DyLight 633 NHS Ester	Kit 5 vials
53051	DyLight 649 Microscale Antibody Labeling Kit DyLight 649 NHS Ester	Kit 5 vials
53057	DyLight 680 Microscale Antibody Labeling Kit DyLight 680 NHS Ester	Kit 5 vials
53061	DyLight 680B Microscale Antibody Labeling Kit DyLight 680B NHS Ester	Kit 5 vials
53059	DyLight 750 Microscale Antibody Labeling Kit DyLight 750 NHS Ester	Kit 5 vials
53063	DyLight 800 Microscale Antibody Labeling Kit DyLight 800 NHS Ester	Kit 5 vials

Antibody Labeling Kits

Contain sufficient reagents to label and purify 3 x 1mg of IgG or similar quantities of other proteins.

In addition to contents listed below, all Antibody Labeling Kits include:

- Reaction Buffer, 1mL
- Spin Columns, 6 each
- Microcentrifuge Collection Tubes, 12 each
- Purification Resin, 5mL



Protocol summary for Thermo Scientific DyLight Antibody Labeling Kits.

Ordering Information

Product #	Description	Pkg. Size
62275	DyLight 350 Antibody Labeling Kit DyLight 350 NHS Ester	Kit 3 vials
53020	DyLight 405 Antibody Labeling Kit DyLight 405 NHS Ester	Kit 3 vials
53024	DyLight 488 Antibody Labeling Kit DyLight 488 NHS Ester	Kit 3 vials
53034	DyLight 549 Antibody Labeling Kit DyLight 549 NHS Ester	Kit 3 vials
53044	DyLight 594 Antibody Labeling Kit DyLight 594 NHS Ester	Kit 3 vials
53046	DyLight 633 Antibody Labeling Kit DyLight 633 NHS Ester	Kit 3 vials
53050	DyLight 649 Antibody Labeling Kit DyLight 649 NHS Ester	Kit 3 vials
53058	DyLight 750 Antibody Labeling Kit DyLight 750 NHS Ester	Kit 3 vials
53056	DyLight 680 Antibody Labeling Kit DyLight 680 NHS Ester	Kit 3 vials
53060	DyLight 680B Antibody Labeling Kit DyLight 680 NHS Ester	Kit 3 vials
53062	DyLight 800 Antibody Labeling Kit DyLight 800 NHS Ester	Kit 3 vials

Fluorescein

Amine-reactive derivatives of fluorescein dye.

NHS-fluorescein and fluorescein isothiocyanate (FITC), two reactive derivatives of fluorescein dye, are used in wide-ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as Western blotting and ELISA. FITC is the base fluorescein molecule functionalized with an isothiocyanate reactive group (–N=C=S), replacing a hydrogen atom on the bottom ring of the structure. This derivative is reactive toward primary amine groups on proteins, peptides and other biomolecules. A succinimidyl-ester functional group attached to the fluorescein core, creating NHS-fluorescein, forms another common derivative that has much greater specificity toward primary amines in the presence of other nucleophiles and a more stable linkage following labeling.

Thermo Scientific Pierce Fluorescein is a mixture of isomers with reactive groups attached at the five and six positions of the bottom ring (Figure 2). The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer.

Fluorescein-5-maleimide and 5-Iodoacetamidofluorescein (5-IAF) are sulfhydryl-reactive derivatives of fluorescein dye. Fluorescein-5-maleimide is the base fluorescein molecule functionalized with a maleimide reactive group by replacing a hydrogen atom on the bottom ring of the structure. 5-IAF is the core fluorescein molecule functionalized with an iodoacetamide group. Both fluorescein derivatives are reactive toward sulfhydryl groups (e.g., reduced cysteine residues) on proteins, peptides and other biomolecules.

A derivative of fluorescein, DyLight 488 Fluor, has been tailored for various chemical and biological applications where greater photostability and fluorescence intensity, pH independence, and a narrower emission spectrum are required.

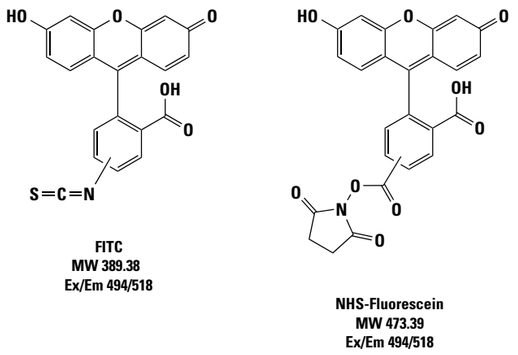
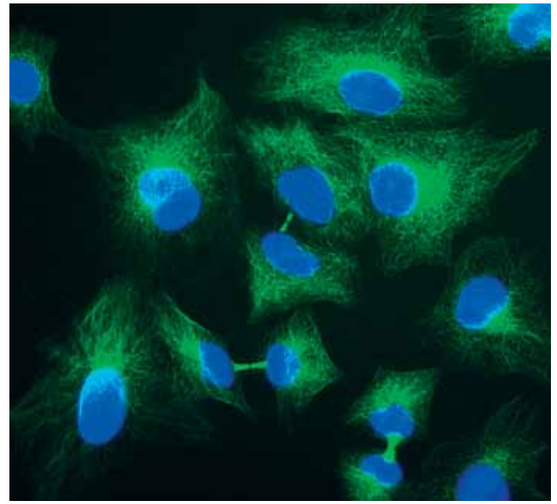


Figure 2. Structures of FITC and NHS-Fluorescein.



The Thermo Scientific Pierce NHS-Fluorescein Antibody Labeling Kit (Product # 53029) produces ideal conjugates for immunofluorescence. A549 cells were fixed with 4% paraformaldehyde (Product # 28906) and permeabilized with 0.1% Surfact-Amps® X-100 (Product # 28314). The cells were then probed with a 0.4µg/mL mouse anti-α-tubulin antibody and 2µg/mL fluorescein-goat anti-mouse secondary antibody. Nuclei were labeled with Hoechst 33342. Images were acquired on Nikon Eclipse TS100 fluorescent microscope using Zeiss AxioCam™ camera and AxioVison™ software.

Ordering Information

Product #	Description	Pkg. Size
46424	FITC (Fluorescein Isothiocyanate)	1g
46425	FITC (Fluorescein Isothiocyanate)	100mg
46409	NHS-Fluorescein	1g
46410	NHS-Fluorescein	100mg
62245	Fluorescein-5-Maleimide	25mg
62246	5-Iodoacetamido-fluorescein (5-IAF)	25mg
53027	FITC Antibody Labeling Kit *Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour.* Includes: FITC Borate Buffer Spin Columns Microcentrifuge Collection Tubes Purification Resin	Kit 3 vials 1mL 6 each 12 each 5mL
53029	Fluorescein Antibody Labeling Kit *Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour.* Includes: NHS Fluorescein Borate Buffer Spin Columns Microcentrifuge Collection Tubes Purification Resin	Kit 3 vials 1mL 6 each 12 each 5mL

Rhodamine

Amine-reactive derivatives of rhodamine dye.

NHS-rhodamine and tetramethylrhodamine isothiocyanate (TRITC), two reactive derivatives of rhodamine dye, are used in wide-ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as Western blotting and ELISA.

TRITC is the base tetramethylrhodamine molecule functionalized with an isothiocyanate reactive group (–N=C=S), replacing a hydrogen atom on the bottom ring of the structure. This derivative is reactive toward amine and sulfhydryl groups on proteins, peptides and other biomolecules. A succinimidyl-ester functional group attached to the tetramethylrhodamine core, creating NHS-fluorescein, forms another common derivative that has much greater specificity toward primary amines in the presence of other nucleophiles and a more stable linkage following labeling. Texas Red Sulfonyl Chloride is a long-wavelength derivative of rhodamine that is modified with sulfonyl chloride for reaction to primary amines.

Thermo Scientific Pierce Rhodamine Dyes are a mixture of isomers with reactive groups attached at the five and six positions of the bottom ring (Figure 3). The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer.

A new derivative of rhodamine, DyLight 549 Fluor, has been tailored for various chemical and biological applications where greater photostability and fluorescence intensity, less pH dependence, and a narrower emission spectrum are required.

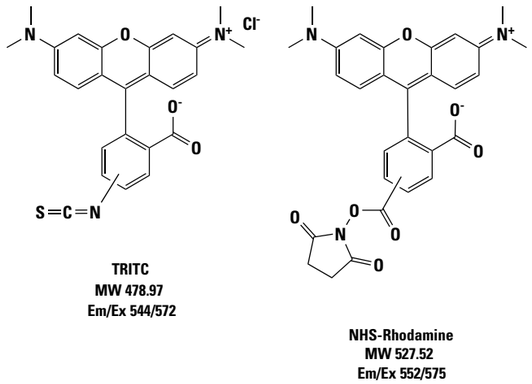
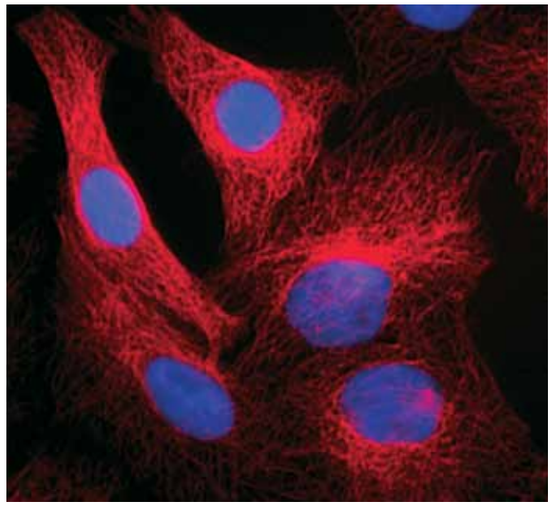


Figure 3. Structures of TRITC and NHS-Rhodamine.



The Thermo Scientific Pierce NHS-Rhodamine Antibody Labeling Kit (Product # 53031) produces ideal conjugates for immunofluorescence. A549 cells were fixed with 4% paraformaldehyde (Product # 28906) and permeabilized with 0.1% Surfact-Amps X-100 (Product # 28314). The cells were then probed with a 0.4µg/mL mouse anti-α-tubulin antibody and 2µg/mL rhodamine-goat anti-mouse secondary antibody. Nuclei were labeled with Hoechst 33342. Images were acquired on Nikon Eclipse TS100 fluorescent microscope using Zeiss AxioCam camera and AxioVison software.

Ordering Information

Product #	Description	Pkg. Size
46112	TRITC (Tetramethylrhodamine Isothiocyanate)	10mg
46406	NHS-Rhodamine	25mg
53031	Rhodamine Antibody Labeling Kit *Efficiently labels and purifies 3 x 1mg of IgG or other protein in about 1 hour.* Includes: NHS Rhodamine Borate Buffer Spin Columns Microcentrifuge Collection Tubes Purification Resin	Kit 3 vials 1mL 6 each 12 each 5mL
46115	Texas Red Sulfonyl Chloride	10 x 1mg

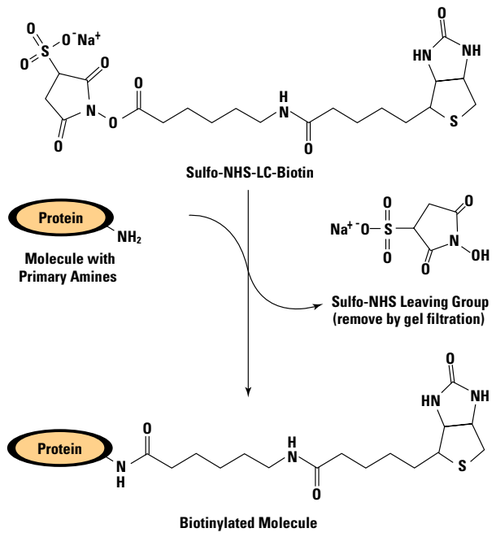
EZ-Link Biotinylation Kits

Everything you need to rapidly and successfully biotinylate purified proteins.

Choose one of these easy-to-use kits and be confident that you have all the tools you need to efficiently biotinylate an antibody or other protein. The Thermo Scientific EZ-Link Kits provide sufficient biotinylation reagent for eight to 10 labeling reactions of 1-10mg of protein each. Each kit also includes an appropriate labeling reaction buffer and 10 Thermo Scientific Zeba Desalt Spin Columns for efficient clean-up of the labeled protein. Finally, the HABA dye and avidin kit components allow one to determine the extent of labeling (i.e., the biotin:protein molar ratio).

Highlights:

- NHS-ester biotins react specifically with primary amines (N-terminus and side chain of lysine residues), resulting in stable amide bond
- Sufficient reagents for 10 labeling reactions (8 reactions for Product # 21455)
- Labels 1-10mg protein in 0.5-2mL per reaction
- Sulfonate form of NHS ester (or the PEG spacer arm for Product # 21455) enhances water solubility of the biotin reagent
- Zeba Desalt Spin Columns (5mL) provide rapid sample clean up and excellent protein recovery (faster than dialysis and gravity-flow gel filtration, and much better protein recovery and desalting efficiency than diafiltration)
- Includes reagents and protocol for determining labeling efficiency



Sulfo-NHS-LC-Biotin reaction scheme.

Sulfo-NHS-Biotin (Product # 21425)

- Shortest spacer arm and simplest biotin reagent
- Adds smallest possible mass to labeled protein

Sulfo-NHS-LC-Biotin (Product # 21435)

- Historically the most popular and widely applied biotin reagent
- Extended spacer arm (compared to Sulfo-NHS-Biotin) minimizes possibility of steric hindrance for avidin or streptavidin binding
- Useful for other applications (e.g., cell-surface labeling) besides labeling purified proteins

Sulfo-NHS-SS-Biotin (Product # 21445)

- Cleavable disulfide bond in spacer arm (can be reduced with DTT or other reducing agent to release the labeled protein avidin or streptavidin in protein interaction or affinity applications)

NHS-PEG$_4$-Biotin (Product # 21455)

- Polyethylene glycol (PEG) spacer arm enhances water solubility
- Hydrophilic PEG spacer confers solubility to labeled protein (i.e., antibodies labeled with this reagent are less likely to aggregate and precipitate during long-term storage than those labeled with other biotin reagents)

Ordering Information

Product #	Description	Pkg. Size
21425	EZ-Link Sulfo-NHS-Biotinylation Kit Includes: EZ-Link Sulfo-NHS-Biotin BupH PBS (pack makes 500mL) Zeba Desalt Spin Columns, 5mL HABA (10mM) Avidin, Affinity-purified	Kit 25mg 1 pack 10 each 1mL 10mg
21435	EZ-Link Sulfo-NHS-LC-Biotinylation Kit Includes: EZ-Link Sulfo-NHS-Biotin Non-reagent contents same as Product # 21425	Kit 25mg
21445	EZ-Link Sulfo-NHS-SS-Biotinylation Kit Includes: EZ-Link Sulfo-NHS-SS-Biotin Non-reagent contents same as Product # 21425	Kit 25mg
21455	EZ-Link NHS-PEG$_4$-Biotinylation Kit Includes: No-Weigh NHS-PEG$_4$-Biotin Non-reagent contents same as Product # 21425	Kit 8 x 2mg

See www.thermoscientific.com/pierce for standalone biotinylation products.

EZ-Link Micro Biotinylation Kits

Just what you need to biotinylate 50-200µg antibody or other protein.

Finally! Biotinylation kits that provide reagents and accessories suited for labeling small amounts of protein. The Thermo Scientific EZ-Link Micro Biotinylation Kits are similar to the kits described on page 46, the reagent in these kits is supplied in a convenient No-Weigh Microtube format and the kits include smaller Thermo Scientific Zeba Desalt Spin Columns that are ideal for small-scale labeling of commercial antibodies and other samples that are available in limited amounts.

Highlights:

- NHS-ester biotins react specifically with primary amines (N-terminus and side chain of lysine residues), resulting in stable amide bond
- Sufficient reagents for eight labeling reactions
- Labels 50-200µg protein in 200-700µL per reaction
- Sulfonate form of NHS ester (or the PEG spacer arm for Product # 21955) enhances water solubility of the biotin reagent
- Zeba Desalt Spin Columns (2mL) provide rapid sample clean up and excellent protein recovery (faster than dialysis and gravity-flow gel filtration, and much better protein recovery and desalting efficiency than diafiltration)
- Micro Biotinylation Kits do NOT include reagents and protocol for determining labeling efficiency because it would use up most of the small amount of labeled sample

Ordering Information

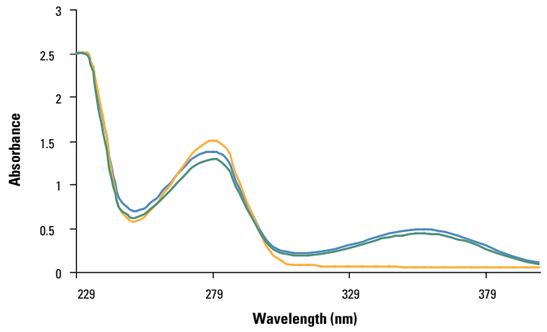
Product #	Description	Pkg. Size
21925	**EZ-Link Micro Sulfo-NHS-Biotinylation Kit** Includes: No-Weigh Sulfo-NHS-Biotin BupH PBS (pack makes 500mL) Zeba Desalt Spin Columns, 2mL	Kit 8 x 2mg 1 pack 10 each
21935	**EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit** Includes: No-Weigh Sulfo-NHS-LC-Biotin Non-reagent contents same as Product # 21925	Kit 8 x 1mg
21945	**EZ-Link Micro Sulfo-NHS-SS-Biotinylation Kit** Includes: No-Weigh Sulfo-NHS-SS-Biotin Non-reagent contents same as Product # 21925	Kit 8 x 1mg
21955	**EZ-Link Micro NHS-PEG$_4$-Biotinylation Kit** Includes: No-Weigh NHS-PEG$_4$-Biotin Non-reagent contents same as Product # 21925	Kit 8 x 2mg

See www.thermoscientific.com/pierce for standalone biotinylation products.

EZ-Link NHS-Chromogenic-Biotinylation Kit

A protein labeling kit with a unique biotinylation reagent.

This convenient kit includes all components needed to label five purified protein samples (1-10mg each) with NHS-Chromogenic-Biotin, a distinctive reagent with a long spacer arm and built-in chromophore for measuring labeling efficiency. Antibodies and other proteins are easily labeled where primary amines occur on their surface. After sample cleanup with a Zeba Desalt Spin Column that is included in the kit, the sample absorbance at 354nm can be used to directly calculate the extent of biotinylation.



Labeling of human IgG with Thermo Scientific EZ-Link NHS-Chromogenic-Biotin. A 1mg/mL human IgG sample in 1mL total volume of phosphate-buffered saline was modified with EZ-Link NHS-Chromogenic-Biotin. Samples were prepared in duplicate and desalted using 5mL Thermo Scientific Zeba Desalt Spin Columns. Note the absorption at 354nm from the covalent addition of the chromogenic biotinylation agent and the minimal loss of protein as illustrated by the 280nm absorption maxima.

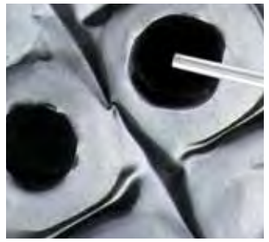
Ordering Information

Product #	Description	Pkg. Size
21625	**EZ-Link NHS-Chromogenic-Biotinylation Kit** Includes: NHS-Chromogenic-Biotin BupH PBS (pack makes 500mL) Zeba Desalt Spin Columns, 5mL DMF (N,N-dimethylformamide)	Kit 10mg 1 pack 5 each 4mL

See www.thermoscientific.com/pierce for standalone biotinylation products.

EZ-Link Solid-Phase Biotinylation Kits

An easier way to biotinylate IgG antibodies.



This innovative antibody-labeling system uses nickel-chelated agarose to temporarily immobilize antibody molecules via their histidine-rich Fc regions. Once held in place on the gel, the antibody can be biotinylated at either sulfhydryl groups (after mild reduction or disulfide bonds) or primary amines. Excess labeling reagent and byproducts are then washed away before recovering the labeled and purified antibody from the gel using a mild imidazole solution. No gel filtration or dialysis are needed. Four kits are available for small (0.1-1mg) or large (1-10mg) antibody samples using either amine-directed (NHS ester) or sulfhydryl-directed (maleimide) labeling reagents.

These kits contain our unique Thermo Scientific No-Weigh™ Single-Dose Microtube Packaging. A single sealed microtube containing 2mg of reagent is reconstituted for each biotinylation. The exclusive No-Weigh™ Packaging allows access to fresh reagent on-demand for each solid-phase biotinylation reaction.

Highlights:

- **Fast labeling and purification** – the entire procedure takes only one hour (two hours for sulfhydryl labeling kits)
- **Easy removal of spent and excess labeling reagent** – simply wash away the reaction byproducts – no need for dialysis or gel filtration
- **No dilution effects** – solid-phase method allows initially dilute antibodies to be recovered in a smaller volume after labeling
- **Optimized protocols** – specific protocols for antibody ensure appropriate level of labeling (2-5 biotins per antibody molecule), minimizing possibility of inactivation caused by overlabeling
- **Sufficient reagents for eight biotin-labeling experiments** – No-Weigh Single-Dose Microtube Packaging ensures that the biotin reagent is fully active for eight separate experiments

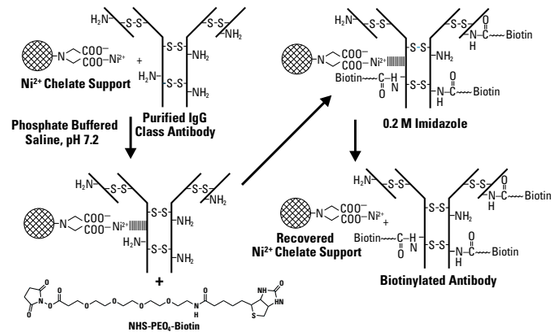
Convenient kit sizes and labeling chemistries available:

Antibody Sample Size	Amine-directed Labeling (NHS-PEG$_4$-Biotin)	Sulfhydryl-directed Labeling (Maleimide-PEG$_2$-Biotin)
0.1-1mg IgG	Product # 21450	Product # 21930
1-10mg IgG	Product # 21440	Product # 21920

Step 1. Immobilize the IgG
a. 1mL Ni-IDA column (for 1-10mg of IgG)
b. SwellGel® Nickel Chelated Disc (for 0.1-1mg IgG)†

Step 2. Add the labeling reagent(s) to the immobilized IgG
a. NHS-PEG$_4$-Biotin for amine-directed reactions
b. TCEP, followed by Maleimide-PEG$_2$-Biotin for sulfhydryl-directed reactions

Step 3. Elute the biotinylated IgG with 0.2M imidazole



Solid-phase protocol summary for labeling amines.

Ordering Information

Product #	Description	Pkg. Size
21440	**EZ-Link NHS-PEG Solid-Phase Biotinylation Kit – Pre-Packed Column** *Biotinylates antibodies and other proteins that bind to the nickel-chelated support provided. A 1mL column biotinylates 1-10mg of antibody and can be re-used 10 times.* Includes: Immobilized Nickel Chelated Column BupH Phosphate Buffered Saline (makes 500mL) No-Weigh NHS-PEG$_4$-Biotin 4M Imidazole Stock Solution	Kit 1mL, pre-packed 1 pack 8 x 2mg 5mL
21450	**EZ-Link NHS-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns** *Biotinylate antibodies and other proteins that bind to the rehydrated nickel-chelated discs provided. Each disc can biotinylate 100-1,000µg of antibody.* Includes: SwellGel Nickel Chelated Discs Mini-Spin Columns Microcentrifuge Tubes (2mL) BupH Phosphate Buffered Saline (makes 500mL) No-Weigh NHS-PEG$_4$-Biotin 4M Imidazole Stock Solution	Kit 10 pack 10 pack 30 pack 1 pack 8 x 2mg 5mL
21920	**EZ-Link Maleimide-PEG Solid-Phase Biotinylation Kit – Pre-Packed Column** *Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. A 1mL column biotinylates 1-10mg of antibody and can be re-used 10 times.* Includes: Bond-Breaker® TCEP Solution, Neutral pH Immobilized Nickel Chelated Column BupH Tris Buffered Saline (makes 500 mL) No-Weigh NHS-PEG$_2$-Biotin 4M Imidazole Stock Solution	Kit 5mL 1mL, pre-packed 1 pack 8 x 2mg 5mL
21930	**EZ-Link Maleimide-PEG Solid-Phase Biotinylation Kit – Mini-Spin Columns** *Reduces and biotinylates IgG class antibodies and other proteins that bind to the nickel-chelated support provided. Each disc can biotinylate 100-1,000µg of antibody.* Includes: SwellGel Nickel Chelated Discs Bond-Breaker TCEP Solution, Neutral pH Mini-Spin Columns Microcentrifuge Tubes (2mL) BupH Tris Buffered Saline (makes 500mL) No-Weigh Maleimide-PEG$_2$-Biotin 4M Imidazole Stock Solution	Kit 10 pack 5mL 10 pack 30 pack 1 pack 8 x 2mg 5mL

See www.thermoscientific.com/pierce for standalone biotinylation products.

Biotin Quantitation Kit

A convenient, accurate method for determining the degree of biotinylation.

Determine the mole ratio of biotin to protein with the Thermo Scientific Pierce Biotin Quantitation Assay. Our easy-to-use No-Weigh Single-dose Packaging and an easy-to-use web-based calculator combine to almost perform the assay for you.

The kit contains HABA and Avidin combined as a single, stable reagent that is easily reconstituted. It also contains a biotinylated HRP positive control. The assay is compatible with both cuvettes and microplates.

The HABA calculator found conveniently at www.thermoscientific.com/pierce simplifies the math. Input your absorbance measurements into the web-based calculator and the mole ratio of biotin to protein is calculated. There's no need to labor over those cumbersome assay equations ever again.

How does this biotinylation assay work?

The HABA4:Avidin complex is at the core of this displacement assay that can estimate the extent of protein biotinylation. HABA dye binds to avidin to form a complex that absorbs strongly at 500nm with an extinction at that wavelength of 35,000 $M^{-1}cm^{-1}$. The assay is based on the decrease in absorbance of the [$(HABA)_4$:Avidin] complex when HABA is displaced from the complex by biotin.

Ordering Information

Product #	Description	Pkg. Size
28005	Pierce Biotin Quantitation Kit *Sufficient materials for estimating the extent of iotinylation of 12 unknowns with 12 positive controls.* Kit contains: No-Weigh HABA/Avidin Premix Biotinylated HRP	Kit 24 tubes 5mg

Fluorescence Biotin Quantitation Kit

Detect down to 750ng of biotinylated IgG.

The highly specific interaction of avidin with biotin (i.e., vitamin H) is useful for designing nonradioactive purification and detection systems. Quantifying the degree of biotinylation is necessary to determine the success of biotinylation and the amount of biotinylated molecule to use for a specific application. The Thermo Scientific Fluorescence Biotin Quantitation Kit contains a premix of fluorescent avidin with HABA and a biocytin standard. This fluorescent method is highly sensitive and accurate and requires only 10µL of sample, whereas the traditional colorimetric method requires 100µL of sample and Supplier I's fluorescent assay requires 50µL of sample. Additionally, our kit more accurately quantitates biotin than Supplier I's fluorescent assay (Figure 4).

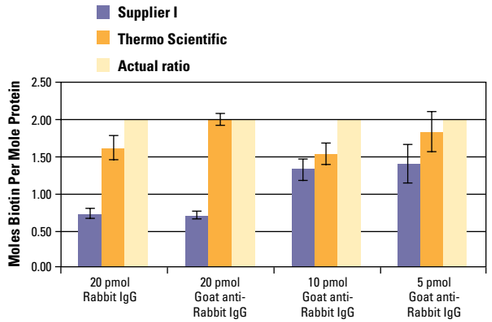


Figure 4. Comparison of results obtained using Thermo Scientific Fluorescence Biotin Quantitation Kit to those generated using a similar kit from Supplier I. Rabbit and goat anti-rabbit IgG samples were spiked with a known amount of Sulfo-NHS-LC-Biotin (Product # 21327), incubated at room temperature for 4 hours and placed at 4°C overnight. Kit protocols were performed according to the manufacturer's instructions. The standard curve for the Supplier I assay was prepared by 1:2 dilution of 1.6µM biocytin. Fifty microliters of each standard and sample were pipetted into a microplate, in triplicate, and 50µL of detection reagent was added. The mixture was incubated for 5 minutes at room temperature and assayed for fluorescence using excitation 485nm and emission 530nm. A linear regression was fit to the linear portion of each standard curve and the equation used to calculate picomoles of biotin per sample. This value was divided by the picomoles of protein in the sample to generate the ratio of moles of biotin per mole of protein.

HABA (4´-hydroxyazobenzene-2-carboxylic acid) is a dye that weakly interacts with avidin and is commonly used in a colorimetric assay to quickly estimate the biotin-to-protein ratio; however, our new fluorescent method is more sensitive and accurate. The premix fluorescent avidin with HABA (Thermo Scientific DyLight Reporter) is added to the solution containing the biotinylated sample. Because of its higher affinity for avidin, biotin displaces the HABA, allowing the avidin to fluoresce.

Ordering Information

Product #	Description	Pkg. Size
46610	Fluorescence Biotin Quantitation Kit *Sufficient reagent to perform 200 microplate assays.* Contains: Phosphate Buffered Saline DyLight Reporter Biocytin Control 200µM	Kit 20 x 3mL 5 vials 100µL

Enzyme Labeling

Maleimide Activation

The heterobifunctional cross-linker SMCC (Product # 22360) and its water-soluble analog Sulfo-SMCC (Product # 22322) have good general utility in preparing immunologically active horseradish peroxidase or alkaline phosphatase conjugates. They are most useful when preparing conjugates of reduced IgG and $F(ab')_2$, because these methods involve the initial step of preparing a maleimide-activated (sulfhydryl-reactive) enzyme derivative. Studies have shown that the two-step maleimide method is superior to glutaraldehyde or *meta*-periodate methods for enzyme conjugation (Figure 5). The maleimide method gives higher yields with less polymerization, producing a conjugate preparation with superior immunoassay characteristics.

Maleimide-activated enzymes can be prepared using the heterobifunctional cross-linker Sulfo-SMCC. This reagent contains an *N*-hydroxy-sulfosuccinimide (Sulfo-NHS) functional group and a maleimide functional group and it is water-soluble due to the presence of the sulfonate ($-SO_3-$) group on the *N*-hydroxysuccinimide ring. The sulfonate group also contributes to the stability of the molecule in aqueous solution. A study of the hydrolysis rate of the maleimide functional group from Sulfo-SMCC showed that it is less prone to hydrolysis to the maleamic acid than the non-sulfonated SMCC. The maleimide groups of Sulfo-SMCC exhibit no decomposition at pH 7 at 30°C within 6 hours. The Sulfo-NHS ester group reacts with primary amines on the enzyme surface to form a stable amide bond. After this first step of conjugation, the enzyme will have maleimide groups on its surface that react optimally toward sulfhydryl groups between pH 6.5 and 7.5 to form stable thioether bonds. Maleimide-mediated conjugation strategies are summarized in Figure 5.

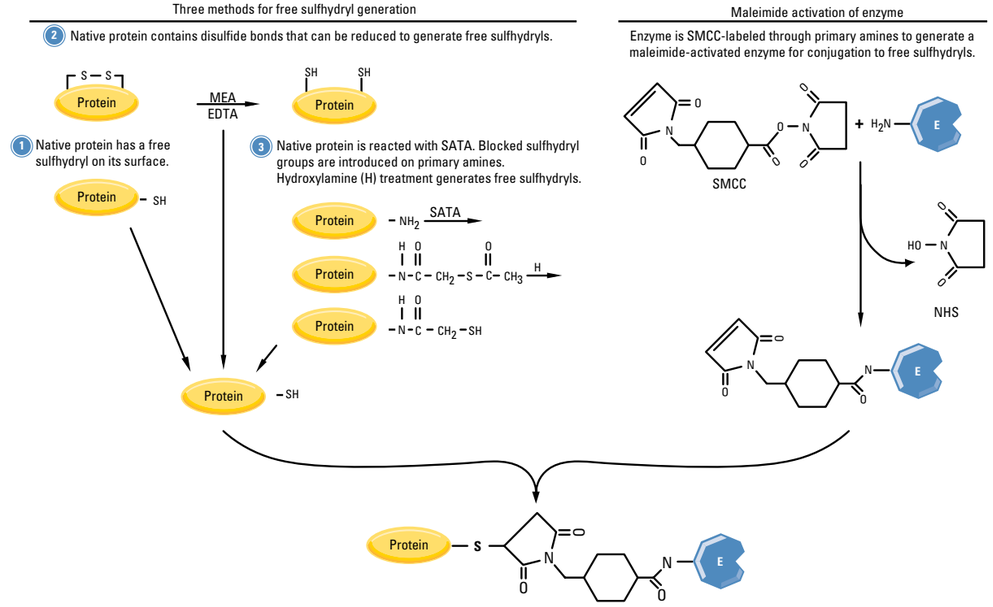


Figure 5. Three strategies for maleimide-mediated conjugation of enzymes.

Two reagents, Mercaptoethylamine•HCl (Product # 20408) and SATA (Product # 26102), are available to produce free sulfhydryls on macromolecules for conjugation to the maleimide-activated enzymes. For labeling antibody molecules, mild reduction with Mercaptoethylamine•HCl (MEA) results in two half-antibody fragments containing free sulfhydryl groups in the hinge region. Labeling in this area is advantageous because it directs the modification away from the antigen-binding region. Native proteins lacking a free sulfhydryl on their surface can be reacted with SATA to generate blocked sulfhydryl groups. The SATA molecule reacts with primary amines via its NHS ester end to form stable amide linkages. The acetylated sulfhydryl group (blocked) is stable until treated with hydroxylamine to generate the free sulfhydryls.

We offer a stable, preactivated enzyme derivative that is reactive toward sulfhydryl (–SH) groups, EZ-Link Maleimide Activated Horseradish Peroxidase (Product # 31485). These products eliminate the first step of the two-step maleimide method, simplifying and facilitating the conjugation protocol, while saving several hours. They can be used to prepare enzyme conjugates directly from proteins, peptides or other ligands that contain a free –SH group. Two reagents, SATA and mercaptoethylamine•HCl, are also included in the kit formats to produce free sulfhydryls on macromolecules for conjugation.

Horseradish Peroxidase (HRP)

Its higher specific enzyme activity makes it the enzyme of choice.

Highlights:

- Superior to alkaline phosphatase and β-galactosidase conjugates because of its higher specific enzyme activity
- Small size (40kDa) allows excellent cellular penetration
- Variety of substrates available
- Ideal in blotting and cytochemistry applications
- Used as the reporter enzyme for Thermo Scientific SuperSignal Chemiluminescent Substrates

References
Cordell, J.L., *et al.* (1984). *J. Histochem. Cytochem.* **32**, 219-229.
Hosoda, H., *et al.* (1987). *Chem. Pharm. Bull.* **35**, 3336-3342.
Passey, R.B., *et al.* (1977). *Clin. Chem.* **23(1)**, 131-139.
Porstmann, B., *et al.* (1985). *J. Immunol. Methods.* **79**, 27-37.
Samoszuk, M.K., *et al.* (1989). *Antibody, Immunoconjugates and Radiopharmaceuticals* **2**, 37-46.
Wordinger, R.J., *et al.* (1987). *Manual of Immunoperoxidase Techniques,* 2nd Edition. Chicago: American Society of Clinical Pathologists Press, pp. 23-24.
Yolken, R.H. (1982). *Rev. Infect. Dis.* **4(1)**, 35-68.

Ordering Information

Product #	Description	Pkg. Size
31490	Horseradish Peroxidase	10mg
31491	Horseradish Peroxidase	100mg

Alkaline Phosphatase (AP)

A highly sensitive enzyme for ELISA and immunohistochemical applications.

Highlights:

- Purified form – ready to conjugate without prior dialysis
- Activity is not affected by exposure to antibacterial agents, such as sodium azide or thimerosal
- Specific activity > 2,000 units/mg
- One unit is defined as the amount that will hydrolyze 1.0 µmol of *p*-nitrophenyl phosphate per minute at 37°C in 1.0M diethanolamine, 0.5mM $MgCl_2$, pH 7.8

Specific activity per mg protein.

Buffer	25°C	37°C
0.1M Glycine, 1.0mM $ZnCl_2$, 1.0mM $MgCl_2$, 6.0mM *p*-Nitrophenyl phosphate, pH 10.4	> 500	> 1,000
1.0M Diethanolamine, 0.5mM $MgCl_2$, 15mM *p*-Nitrophenyl phosphate, pH 9.8	> 1,000	> 2,000

References
Bulman, A.S. and Heyderman, E. (1981). *J. Clin. Pathol.* **34**, 1349-1351.
Cordell, J.L., *et al.* (1984). *J. Histochem. Cytochem.* **32**, 219-229.
Yolken, R.H. (1982). *Rev. Infect. Dis.* **4**, 35-68.

Ordering Information

Product #	Description	Pkg. Size
31391	Alkaline Phosphatase Calf intestinal. Supplied in Tris Buffer, pH ~7 Triethanolamine, 1mM $MgCl_2$, 3M NaCl, pH 7.6	20mg
31392	Alkaline Phosphatase	100mg

EZ-Link HRP Enzyme Labeling Kits and Reagents

Thermo Scientific EZ-Link Pre-Activated HRP makes it easy to convert almost any protein to a detection reagent. Simple protocols with each EZ-Link Kit or Activated Enzyme turn a chemical chore into a painless process.

Horseradish peroxidase (HRP) is the most common enzyme used for immunoassay detection systems. This enzyme catalyzes reactions with substrates to form soluble color responses or colored precipitates, or to generate the chemical emission of light (chemiluminescence). Enzyme conjugates make stable assay reagents and can be stored for long periods at -20°C.

In one hour at room temperature, you can conjugate:

- HRP to a primary amine group ($–NH_2$) with the EZ-Link Plus Activated Peroxidase Kit
- HRP to a free sulfhydryl (–SH) group with the EZ-Link Maleimide Activated HRP Kit

EZ-Link Maleimide Horseradish Peroxidase

Make quick and easy enzyme conjugates.

The Thermo Scientific EZ-Link Maleimide Activated HRP Kit can be used to directly prepare antibody conjugates via sulfhydryl groups. This kit eliminates the first step of the two-step maleimide method, making conjugate preparation much simpler. The kit is supplied with a stable, preactivated HRP.

The HRP conjugates can be purified by gel filtration chromatography, ultrafiltration or dialysis, depending upon the size of the conjugated protein. Alternatively, use Thermo Scientific Pierce Conjugate Purification Kit (Product # 44920) to separate unreacted enzyme from the conjugate.

Highlights:

- Prepare HRP conjugates from proteins that contain a free sulfhydryl
- Includes 2-mercaptoethylamine to generate free sulfhydryls from disulfide bonds
- Includes SATA to add free sulfhydryls to lysine residues

References
Choi, J.Y., *et al.* (2002). *J. Biol. Chem.* **277**, 21630-21638.
Seo, Y.R., *et al.* (2002). *Proc. Natl. Acad. Sci. USA* **99**, 14548-14553.
Yoo, J.H., *et al.* (2004). *J. Biol. Chem.* **279**, 848-858.

Ordering Information

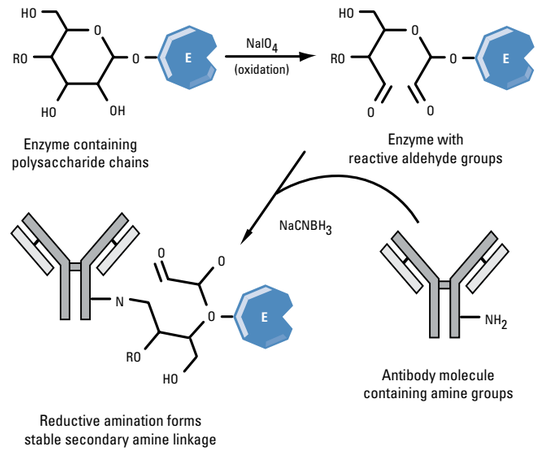
Product #	Description	Pkg. Size
31485	EZ-Link Maleimide Activated Horseradish Peroxidase	5mg
31494	EZ-Link Maleimide Activated Horseradish Peroxidase Kit	Kit
	Includes: EZ-Link Maleimide Activated Horseradish Peroxidase	5mg
	Activated Horseradish Peroxidase Conjugation Buffer	20mL
	2-Mercaptoethylamine•HCl	6mg
	SATA	2mg
	Dimethylformamide	1mL
	Hydroxylamine•HCl	5mg
	Polyacrylamide Desalting Column	1 x 10mL
23460	Protein-Coupling Handle Addition Kit	Kit
	Includes: SATA	2mg
	Hydroxylamine•HCl	5mg
	Conjugation Buffer Stock (10X)	20mL
	BupH Pack PBS	1 pack
	Dimethylformamide (DMF)	1mL
	Dextran Desalting Column	1 x 5mL
	Column Extender	1
	Ellman's Reagent (DTNB)	2mg
	Cysteine•HCl H_2O	20mg

Periodate

Glycoproteins such as horseradish peroxidase and glucose oxidase and most antibody molecules can be activated for conjugation by treatment with periodate. Oxidizing polysaccharide residues in a glycoprotein with sodium periodate provides a mild and efficient way of generating reactive aldehyde groups for subsequent conjugation with amine- or hydrazide-containing molecules via reductive amination. Some selectivity of monosaccharide oxidation may be accomplished by regulating the concentration of periodate in the reaction medium. In the presence of 1mM sodium periodate, sialic acid groups are specifically oxidized at adjacent hydroxyl residues, cleaving off two molecules of formaldehyde and leaving one aldehyde group. At higher concentrations of sodium periodate (10mM or greater), other sugar residues will be oxidized at points where adjacent carbon atoms contain hydroxyl groups. This reaction should be performed in the dark to prevent periodate breakdown and for a limited period of time (15-30 minutes) to avoid loss of enzymatic activity.

Crosslinking with an amine-containing protein takes place under alkaline pH conditions through the formation of Schiff base intermediates. These relatively labile intermediates can be stabilized by reduction to a secondary amine linkage with sodium cyanoborohydride. Reductive amination has been done using sodium borohydride or sodium cyanoborohydride; however, cyanoborohydride is the better choice because it is more specific for reducing Schiff bases and will not reduce aldehydes. Small blocking agents such as lysine, glycine, ethanolamine or Tris can be added after conjugation to quench any unreacted aldehyde sites. Ethanolamine and Tris are the best choices for blocking agents because they contain hydrophilic hydroxyl groups with no charged functional groups.

The pH of the reductive amination reaction can be controlled to affect the efficiency of the cross-linking process and the size of the resultant antibody-enzyme complexes formed. At physiological pH, the initial Schiff base formation is less efficient and conjugates of lower molecular weight result. At more alkaline pH (i.e., pH 9-10), Schiff base formation occurs rapidly and with high efficiency, resulting in conjugates of higher molecular weight and greater incorporation of enzyme when oxidized enzyme is reacted in excess. Low molecular weight conjugates may be more optimal for immunohistochemical staining or blotting techniques in which penetration of the complex through membrane barriers is an important consideration. Washing steps also more effectively remove excess reagent if the conjugate is of low molecular weight, thus maintaining low background in an assay. By contrast, conjugates of high molecular weight are more appropriate for ELISA procedures in a microplate format, where high sensitivity is important and washing off excess conjugate is not a problem.



Conjugation scheme for periodate oxidation and subsequent reductive amination.

EZ-Link Plus Activated Peroxidase Kit

Amine-reactive HRP with the highest conjugation yields.

Thermo Scientific EZ-Link Plus Activated Peroxidase is an amine-reactive HRP derivative that provides coupling efficiencies of greater than 95%. Other amine-reactive chemistries, like glutaraldehyde, tend to polymerize and produce lower amounts of viable conjugate. Sugar residues present on HRP have been oxidized with periodate to produce aldehydes that react with primary amines.

Our EZ-Link Plus Activated Peroxidase is mixed with the protein to be coupled at a pH compatible with your protein or antibody. After incubating the reaction for one to two hours, the conjugate linkage is reduced and the activated HRP quenched with ethanolamine. The conjugate is purified by desalting or by dialysis. If you are preparing an antibody-HRP conjugate, use the Thermo Scientific Pierce Conjugate Purification Kit (Product # 44920) to separate unreacted enzyme from the conjugate.

Highlights:

- Reacts with readily accessible primary amines to form a covalent secondary amine bond
- Can be stored for at least 12 months at -20°C
- Consistent conjugation yields reliable conjugates
- Enzyme activity is 120-200 units/mg

Suggested antibody-HRP dilutions for colorimetric substrates.

Technique	Working Range
Immunohistochemistry	1:10-1:100
Immunoblotting	1:2,000-1:10,000
EIA	1:5,000-1:20,000

Active aldehyde reaction scheme.

EZ-Link Plus Activated Peroxidase References
Glover, L., *et al.* (2002). *Eur. J. Biochem.* **269**, 4607-4616.
Nawa, M., *et al.* (2000). *Clin. Diagn. Lab. Immunol.* **7**, 774-777.
Völkel, T., *et al.* (2001). *Protein Eng.* **14**, 815-823.

Ordering Information

Product #	Description	Pkg. Size
31487	EZ-Link Plus Activated Peroxidase (Periodate Activated)	1mg
31488	EZ-Link Plus Activated Peroxidase (Periodate Activated)	5 x 1mg
31489	EZ-Link Plus Activated Peroxidase (Periodate Activated)	Kit
	Includes: EZ-Link Plus Activated Peroxidase	5 x 1mg
	Sodium Cyanoborohydride Solution	1 x 0.5mL
	Quenching Buffer	25mL
	BupH Phosphate Buffered Saline Pack	500mL
	BupH Carbonate Buffer Pack	500mL

Glutaraldehyde

Another method for conjugation uses glutaraldehyde, one of the oldest homobifunctional crosslinking reagents used for protein conjugation. It reacts with amine groups to create crosslinks by one of several routes. Under reducing conditions, the aldehydes on both ends of glutaraldehyde will couple with amines to form secondary amine linkages. The reagent is highly efficient at protein conjugation but has a tendency to form various high-molecular weight polymers, making results difficult to reproduce.

EZ-Link Activated Peroxidase and Antibody Labeling Kit

The easy way to make HRP-antibody conjugates.

Primary amines on the Thermo Scientific EZ-Link Activated HRP have been converted to active aldehydes. This chemistry can be used to couple HRP to primary amines on another protein such as an antibody. The EZ-Link Activated Peroxidase Kit is designed to purify HRP-antibody conjugates by using Immobilized Protein A/G to separate unreacted enzyme from the antibody conjugate. Using EZ-Link Activated HRP produces an HRP-antibody conjugate in which both the enzyme activity and the antigen-binding activity are preserved.

Highlights:

- Reacts with primary amines to form a covalent amide bond
- No reduction step is necessary to secure the linkage
- Can be stored for at least 12 months at -20°C
- One mg produces about 0.5mL of conjugate with a working dilution of 1:1,000 when coupled to a high titer antibody
- Enzyme activity is > 200 units/mg

EZ-Link Activated Peroxidase References
Sandt, C.H. and Hill, C.W. (2001). *Infect. Immun.* **69**, 7293-7303.
Turpin, E.A., *et al.* (2003). *J. Clin. Microbiol.* **41**, 3579-3583.

Ordering Information

Product #	Description	Pkg. Size
31496	EZ-Link Activated Peroxidase (Glutaraldehyde Activated)	1mg
31495	EZ-Link Activated Peroxidase (Glutaraldehyde Activated)	5mg
31497	EZ-Link Activated Peroxidase Antibody Labeling Kit (Glutaraldehyde Activated)	Kit
	Includes: EZ-Link Activated Peroxidase	5mg
	Conjugation Buffer	50mL
	Lysine	250mg
	Immobilized Protein A/G Column	0.5mL
	Gentle Ag/Ab Binding Buffer	200mL
	Gentle Ag/Ab Elution Buffer	200mL

Storing Enzyme Conjugates

A variety of Thermo Scientific reagents are available to help preserve enzyme conjugate activity. Typically, conjugates are aliquoted in 50-100µL increments using purified ethylene glycol (Product # 29810) as a preservative for -20°C storage. Conjugates can maintain activity for up to two years. An alternative to aliquoting is to use Thermo Scientific Pierce Peroxidase Conjugate Stabilizer (Product # 31503), diluting the conjugate 1:1 in the stabilizer and storing it at -20°C for up to one year as a stock solution. Pierce Peroxidase Stabilizer/Diluents (Product #s 37548 and 37552) allow peroxidase conjugates to be reconstituted and stored at 4°C as a 1:1,000 dilution or a 1:100,000 dilution stock solution.

Conjugate Stabilizers

Ordering Information

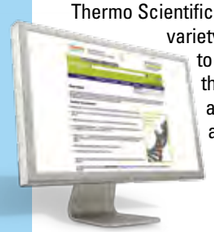
Product #	Description	Pkg. Size
37548	Pierce Peroxidase Conjugate Stabilizer/Diluent	200mL
37552	Pierce Peroxidase Conjugate Stabilizer/Diluent	1L
31503	Pierce Peroxidase Conjugate Stabilizer	25mL
29810	Ethylene Glycol (50% aqueous solution)	200mL

Antibodies & Custom Antibody Services

Don't have the time to produce the antibodies you need? Then consider the extensive line of Thermo Scientific Pierce Antibodies and Custom Antibody Services.

Antibodies

Over 30,000 antibodies in 42 research areas.



Thermo Scientific Pierce Antibodies are developed for a wide variety of application needs. Our website enables you to easily search by protein target and then filter by the specific assays you are interested in. All of our antibodies are validated in the stated applications and are guaranteed to perform.

Applications

- Agglutination
- Competition Assay
- ChIP Assay
- Cytotoxicity Assay
- Control
- ELISA
- Electron Microscopy
- FACS
- Functional Assay
- Gel Shift
- Hemagglutination Assay
- Inhibition Assay
- Immunocytochemistry
- Immunodiffusion
- Immunofluorescence
- Immunohistochemistry
- Immunohistochemistry (Frozen)
- Immunohistochemistry (Paraffin)
- Immunohistochemistry (Paraffin, Frozen)
- Infection
- Immunoprecipitation
- Immunoradiometric Assay
- Radioimmune Assay
- Western Blot

Visit www.thermoscientific.com/Ab for a complete listing.

Custom Antibody Services

Design and produce better custom antibodies.

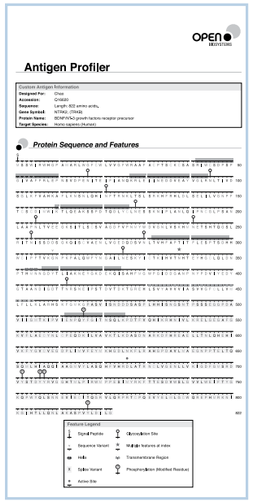
The Thermo Scientific Open Biosystems Custom Antibody Development Service leverages our experience in making more than 18,500 antibodies to peptides and recombinant proteins. Our proprietary antigen design tools, including the Thermo Scientific Antigen Profiler Software (below), and targeted antigen display produces more robust antibodies that perform better in your targeted assays.

When you initiate a custom antibody project with us we provide you access to our online project management tool. This secure account gives you easy access to project information and allows you to provide specific instructions for your projects.

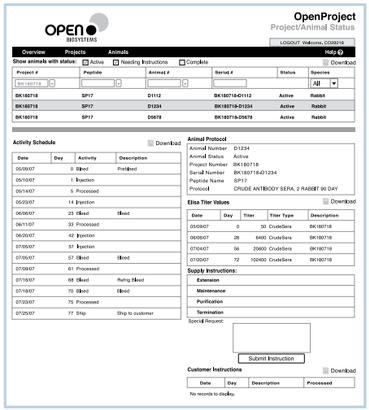
Custom Antibody Options

	Recombinant Protein Polyclonal	Anti-Peptide Polyclonal	Monospecific Polyclonal	Phospho-Specific Polyclonal	Anti-Peptide Monoclonal	Recombinant Protein Monoclonal
Advanced Antigen Design with Antigen Profiler™ Software	✔	✔	✔	✔	✔	✔
Peptide Synthesis	NA	✔	✔	✔	✔	NA
Depleting Peptide or Control Peptide	NA	NA	✔	✔	Optional	NA
Protein Expression	Optional	NA	NA	NA	NA	✔
Animal Immunization Protocol	✔	✔	✔	✔	✔	✔
ELISA Titration	✔	✔	✔	✔	✔	✔
Affinity Purification and Depletion	Optional	Optional	✔	✔	NA	NA
Fusions and Cloning	NA	NA	NA	NA	✔	✔
Monoclonal Production and Purification	NA	NA	NA	NA	Optional	Optional
Complete Online Project Tracking	✔	✔	✔	✔	✔	✔

For more information on Thermo Scientific Open Biosystems Custom Antibody Services, please visit www.thermoscientific.com/openbio.



Thermo Scientific Antigen Profiler Software provides the tools to develop better antibodies. The online software allows you to examine comparative antigenicity based on *in vivo* titer data from over 13,000 peptide antibodies and to identify important structural motifs to produce high specificity antibodies. The software also includes a peptide tutorial to aid in synthesizing soluble peptides and curated BLAST analysis for specificity determination. A sample report is shown.



Example of online project tool.

Handbooks

Thermo Scientific Pierce Antibody Catalog

With the addition of ABR-Affinity BioReagents to the Thermo Scientific family of products, you now have access to over 30,000 polyclonal and monoclonal antibodies in 42 research areas. These products are a perfect complement to many Thermo Scientific Pierce Protein Research products.

- Over 30,000 polyclonal and monoclonal antibodies in 42 research areas ... and growing!
- Numerous secondary antibodies, affinity-tagged antibodies, recombinant proteins and peptides
- An extensive line of antibodies to many Thermo Scientific Open Biosystems cDNA, ORF and shRNA clones
- siRNA-validated antibodies...proven specificity especially for Western blot detection
- Research areas include cancer biology, cytokines, heat shock protein, immunology, ion transport, neurobiology, signal transduction and more

Visit www.thermoscientific.com/abr for more information.

Thermo Scientific Custom Antibody and Proteomics Brochure

Learn how you can obtain better antibodies. The Thermo Scientific Open Biosystems Custom Antibody and Biomarker Development Service leverages our experience in making more than 18,500 antibodies to peptides and recombinant proteins, our proprietary antigen design tools and targeted antigen display to produce more robust antibodies that perform better in your targeted assays.

Our suite of world-class services includes:

- Custom polyclonal antibodies
- Custom hybridoma (monoclonal) development
- Custom peptide synthesis
- Custom protein expression
- Custom monoclonal production

Visit www.thermoscientific.com/openbiosystems for more information.

Avidin-Biotin Technical Handbook

This 49-page guide brings together everything needed to biotinylate cell-surface proteins, purify a biotinylated target, detect a biotinylated antibody and perform many other applications. It includes dozens of references along with protocols, troubleshooting tips, selection guides and a complete listing of available tools.

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Life Science Research
Contact Information

Belgium and Europe,
the Middle East
and Africa Distributors
Tel: +32 53 85 71 84

France
Tel: 0 800 50 82 15

The Netherlands
Tel: 076 50 31 880

Germany
Tel: 0228 9125650

United Kingdom
Tel: 0800 252 185

Switzerland
Tel: 0800 56 31 40

Email: perbio.euromarketing@thermofisher.com
www.thermoscientific.com/perbio

United States
Tel: 815-968-0747 or 800-874-3723
Customer Assistance E-mail:
Pierce.CS@thermofisher.com
www.thermoscientific.com/pierce

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