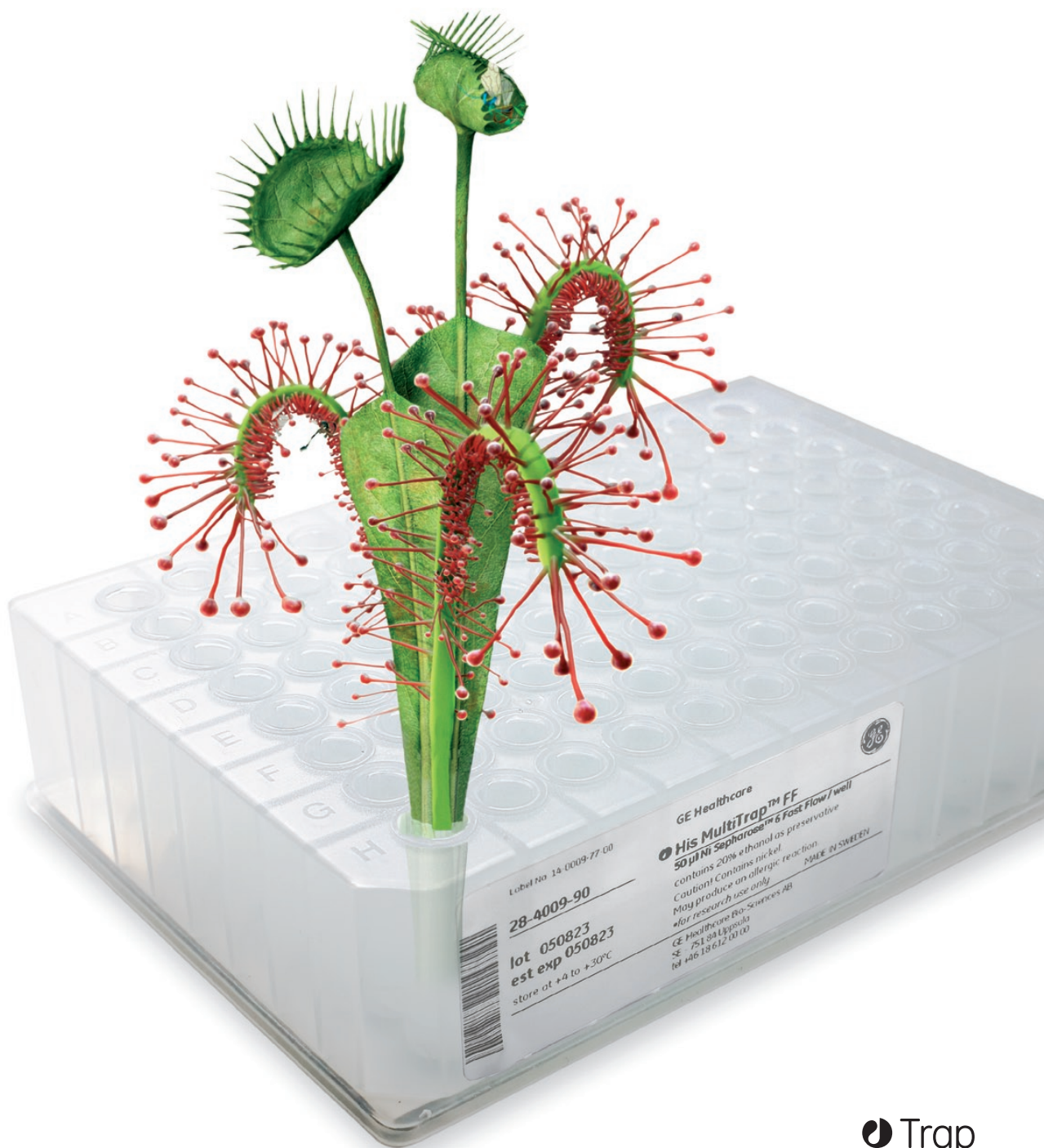


MultiTrap 96-well plates

Applications and guidelines



MultiTrap helps you to work in parallel right from the start

When you need to prepare several protein samples at the same time, handling things in sequence becomes a major headache. Time and labor costs, not to mention reproducibility, become issues. We developed MultiTrap™ 96-well plates to solve these problems.

Specifically, MultiTrap addresses the growing demand for expression screening of large numbers of protein variants and mutants, for developing enrichment methods, for capturing and purifying proteins, and for initial mapping of protein structure and function. MultiTrap also supports protein interaction studies in systems biology.

MultiTrap plates are prepacked 96-well filter plates that can be used manually or in automated systems, and are designed for high-throughput applications in the following areas :

- Expression screening of proteins
- Optimization of expression
- Optimization of enrichment conditions prior to analysis
- Parallel screening of antibodies
- Parallel screening of tagged proteins
- Parallel immunoprecipitation (Pull down)
- Sample preparation - desalting and buffer exchange

This guide helps you to get the most out of MultiTrap, by presenting a number of typical applications, as well as guidelines to help you get great results right from the start.



Reproducible expression screening of tagged proteins

In studies investigating drug targets, automation plays an essential role in screening the expression of tagged proteins as it provides both reproducibility and throughput.

To evaluate the reproducibility when automating purification, six different histidine-tagged proteins, expressed in individual *E. coli* samples were screened six times, and then captured and purified on His MultiTrap HP.

We used a vacuum protocol on Hamilton MicroLab STAR™ Liquid Handling Workstation equipped with MicroLab STAR Basic Vacuum System. All eluates were analyzed by SDS-PAGE. A one-step elution with His MultiTrap HP gave highly pure proteins with excellent reproducibility*.

96-well plate: His MultiTrap HP

Sample: ~500 µl/well of bacterial extract of six different *E. coli* samples expressing histidine-tagged proteins of diverse molecular weight. Only four of the six histidine-tagged proteins were expressed at significant levels. No incubation.

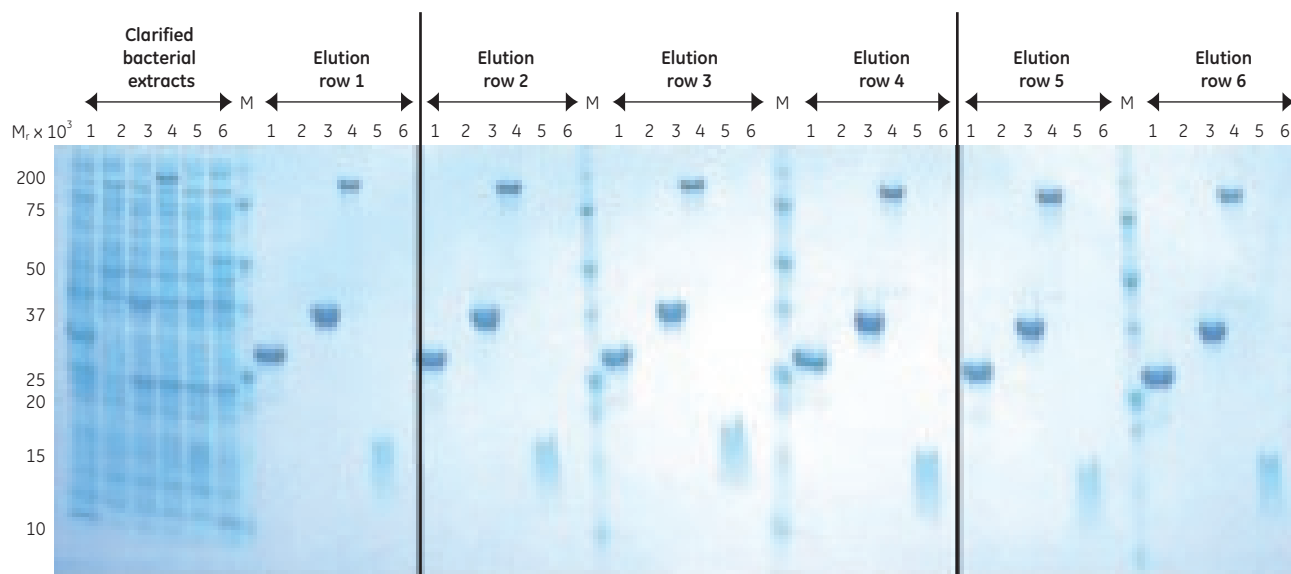
Equilibration/binding buffer: 50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.4

Wash buffer: 50 mM Tris-HCl, 500 mM NaCl, 25 mM imidazole, pH 7.4

Elution buffer: 50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.4

High-throughput method: Automated vacuum

* GST MultiTrap is also available for expression screening of GST-tagged proteins.



Summary

Expression screening of histidine-tagged proteins using MultiTrap with an automated protocol allowed processing of 96 samples in less than 30 min with high purity and reproducibility.

Acknowledgement: B. Gallet, M. Noirclerc-Savoye and T. Vernet RoBioMol/Laboratory for Macromolecular Engineering, Institut de Biologie Structurale CEA-CNRS-UJF, Grenoble, France

Efficient screening of buffer conditions

To achieve optimal recovery of a target protein, it is important to fine-tune a number of buffer conditions, such as buffering chemistry, pH, and salt content. This application presents an solution to this complex, multivariable screening of buffer conditions to achieve optimal purification of the target protein.

Here, the optimal binding buffer was identified for a histidine-tagged transcription factor involved in the development of dopamine neurons.

Ninety-six buffer conditions were screened simultaneously on a single His MultiTrap FF to find optimal purification conditions using a Tecan™ Freedom EVO™ liquid handling station equipped with a centrifuge.

Eight buffer solutions ranging from pH 6.0 to 8.5 were screened. For each of them, the concentrations of sodium chloride, glycerol and β -mercaptoethanol were varied. SDS-PAGE was used to determine the recovery of the target protein from the lysate.

The highest yield was achieved using the buffer containing 25 mM Tris, 100 mM NaCl and 10% glycerol, pH 8.5 (see arrow). These conditions could be applied subsequently to purification on ÄKTAexpress™.

96-well plate: His MultiTrap FF

Sample: ~500 μ l/well of bacterial extract of *E.coli* sample expressing histidine-tagged Nurr1 ligand binding domain (M_r , 26 000)

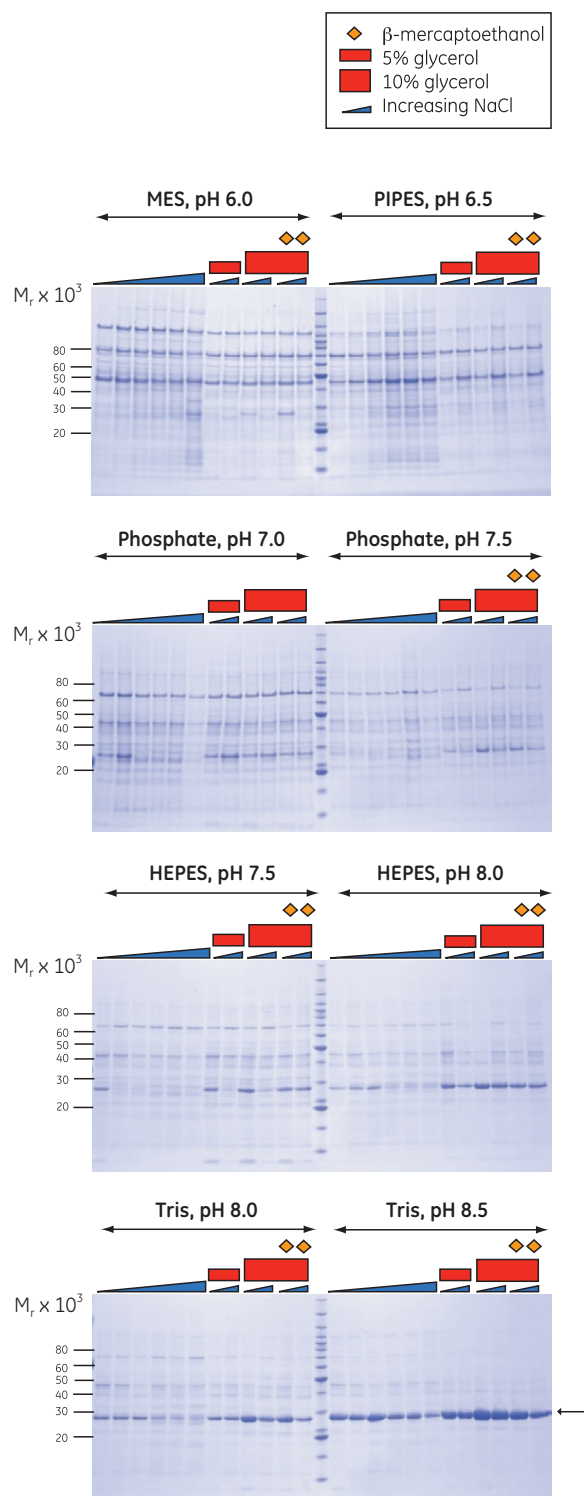
Sample preparation: Chemical and enzymatic lysis

Lysis/binding buffer: Buffer matrix with 1x Bugbuster™ and 25 U/ml Benzonase, 1 kU/ml rLysozyme™ and 2x Complete™ EDTA-free protease inhibitors

Wash buffer: Buffer matrix with 50 mM imidazole

Elution buffer: Buffer matrix with 500 mM imidazole

High-throughput method: Automated centrifugation



Summary

A parallel buffer screening method enables efficient identification of optimal conditions for a specific target protein, which can then be used for protein scale-up.

Acknowledgement: Ruth Steele and Dr. Bruce Grasberger, Johnson and Johnson, Exton, USA

Optimizing solubility of membrane proteins

When purifying membrane proteins, the choice of detergent is highly important for achieving good yields, and ultimately, for recovering proteins with retained activity. Screening for the right detergent characteristics to solubilize membrane proteins is a great help towards the best possible outcome in downstream scale-up work.

In this example, eight detergents were screened for their effect on the solubility of histidine tagged membrane proteins. Results from purification screening of four membrane proteins, on His MultiTrap FF are shown below. The samples were processed through a centrifuge for the capture and purification of the proteins.

96-well plate: His MultiTrap FF

Samples: 100 µl/well of four *E.coli* lysates expressing histidine-tagged EM05 (a putative transferase), EM08 (a regulatory protein), EM29 (a GlpG protein) and EM43 (a cation transporter)

Sample preparation: Chemical and freeze/thaw lysis

Lysis buffer: 20 mM sodium phosphate, 100 mM NaCl, 20 mM imidazole, 0.5 mM tris(2-carboxylethyl)phosphine (TCEP), 5 u/ml benzonase, 1 mg/ml lysozyme, EDTA-free protease inhibitor cocktail, 1% to 2% detergent, pH 7.4

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, 0.5 mM TCEP, 1% to 2% detergent, pH 7.4

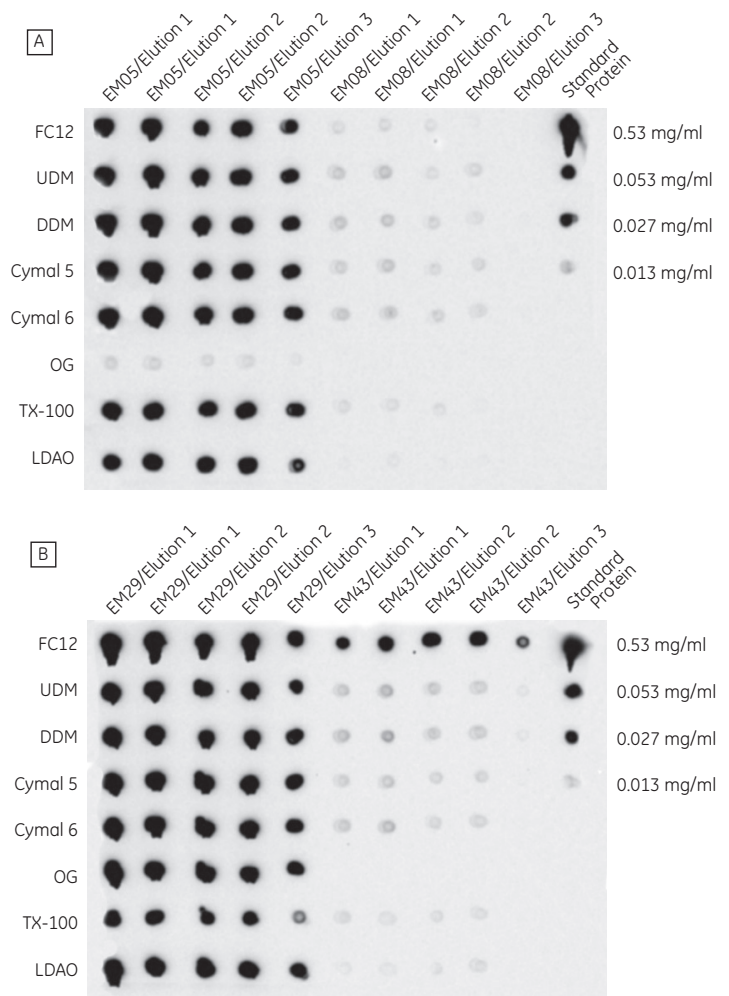
Wash buffer: 20 mM sodium phosphate, 500 mM NaCl, 40 mM imidazole, 0.5 mM TCEP, 0.03% dodecyl maltoside (DDM), 1% to 2% detergent, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, 0.5 mM TCEP, 0.03% DDM, 1% to 2% detergent, pH 7.4

Detergents: 1% fos-choline 12 (FC12), 1% undecyl maltoside (UDM), 1% DDM, 1% Cymal™ 5, 1% Cymal 6, 2% octyl glycoside (OG), 1% Triton™ X-100 (TX-100), 1% lauryl dimethylamine oxide (LDAO)

High-throughput method: Manual centrifugation

Data evaluation: Dot-blot analysis on nitrocellulose membrane; histidine-tagged proteins were detected using INDIA HisProbe™ - HRP Western blotting probe



Summary

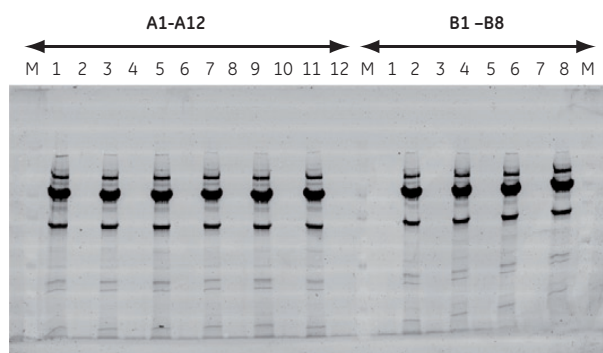
A number of detergents can easily be screened using either His MultiTrap FF or His MultiTrap HP to optimize the solubility of histidine-tagged membrane proteins.

Acknowledgment: Said Eshaghi, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

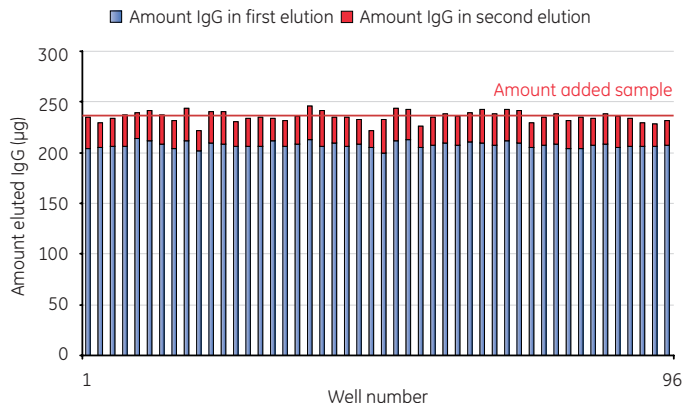
Parallel antibody screening without cross-contamination

Protein A MultiTrap HP and Protein G MultiTrap HP are versatile tools that can be used for a number of operations such as antibody screening or purifying antibodies from a variety of sources. They offer highly reproducible well-to-well performance with no detectable cross-contamination. Excellent well-to-well reproducibility is shown with a simple setup on a Tecan Freedom EVO liquid handling station equipped with Te-VacS™ and Magellan™.

Human monoclonal IgG was purified on Protein A HP MultiTrap dispensed in a chessboard pattern, with neighboring wells left empty. Every well was analyzed for possible cross-contamination. After elution, yields were calculated by measuring absorbance at 280 nm and fractions were analyzed by SDS-PAGE. Highly reproducible purification was achieved with a relative standard deviation of 1.4%, with no detectable antibody in the empty wells.



Deep Purple™ stained SDS-PAGE (non-reduced) gel of eluted monoclonal IgG from first elution. Samples were taken from wells A1-A12 and B1-B8. M = molecular weight marker. In this test, every second well in the MultiTrap was empty, and this analysis clearly demonstrates that no cross-contamination was detectable.



The total yield of IgG among wells varied with a standard deviation of less than 2%.

96-well plate: Protein A HP MultiTrap

Sample: 200 µl/well of pure monoclonal human IgG

Binding buffer: 20 mM sodium phosphate, pH 7.0

Wash buffer: 20 mM sodium phosphate, pH 7.0

Neutralizing buffer: 1 M Tris-HCl, pH 9.0

Elution buffer: 0.1 M glycine-HCl, pH 2.7

High-throughput method: Manual centrifugation

Analysis and data evaluation: Absorbance measurement at 280nm to calculate RSD% for yield, SDS-PAGE with Deep Purple staining and scanning of the gel using Ettan™ DIGE Imager.

The scanned gel was analyzed using ImageQuant™ TL software

	1	2	3	4	5	6	7	8	9	10	11	12
A	S	E	S	E	S	E	S	E	S	E	S	E
B	E	S	E	S	E	S	E	S	E	S	E	S
C	S	E	S	E	S	E	S	E	S	E	S	E
D	E	S	E	S	E	S	E	S	E	S	E	S
E	S	E	S	E	S	E	S	E	S	E	S	E
F	E	S	E	S	E	S	E	S	E	S	E	S
G	S	E	S	E	S	E	S	E	S	E	S	E
H	E	S	E	S	E	S	E	S	E	S	E	S

Samples are applied in a chessboard pattern, where S indicates sample present and E indicates an empty well. No cross-contamination was detected between wells.

Summary

The automated protocol for antibody screening showed a high level of robustness with minimal deviation in the yield of protein and no cross-contamination between the samples.

Protein enrichment for biomarker research

Searching for biomarkers requires extensive research efforts. Proteins of interest are in low abundance, and detection is impaired by the presence of highly abundant proteins. To obtain statistically reliable data, large numbers of biological and experimental replicates may be needed. Parallel methods for protein enrichment can improve the outcome dramatically.

Protein G HP MultiTrap is designed for enrichment of proteins of interest from cell lysates and biological fluids by immunoprecipitation. This procedure is useful in enhancing the signal in the subsequent analysis, for example by mass spectrometry.

Identifying proteins in complex mixtures

Human serotransferrin (hTf) was added to an *E. coli* lysate, making up 0.15% of the protein content. Prior to enrichment, the only proteins detected were highly abundant *E. coli* proteins. After enrichment, about 50 proteins that were otherwise undetectable were identified with confidence. hTf was not detected in the starting material, but was the major protein hit in the enriched sample (enrichment from 0.15% to about 15% purity).

96-well plate: Protein G HP MultiTrap

Sample: 200 μ l/well of clarified *E. coli* extract containing 7.5 μ g/ml human serotransferrin, total protein conc. 5 mg/ml

Antibody: Polyclonal rabbit anti-human transferrin

Binding buffer: 50 mM Tris, 150 mM NaCl, pH 7.5

Wash buffer: 50 mM Tris, 150 mM NaCl, 2 M urea, pH 7.5

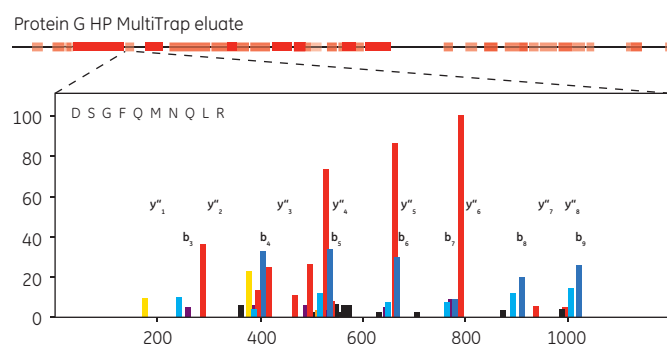
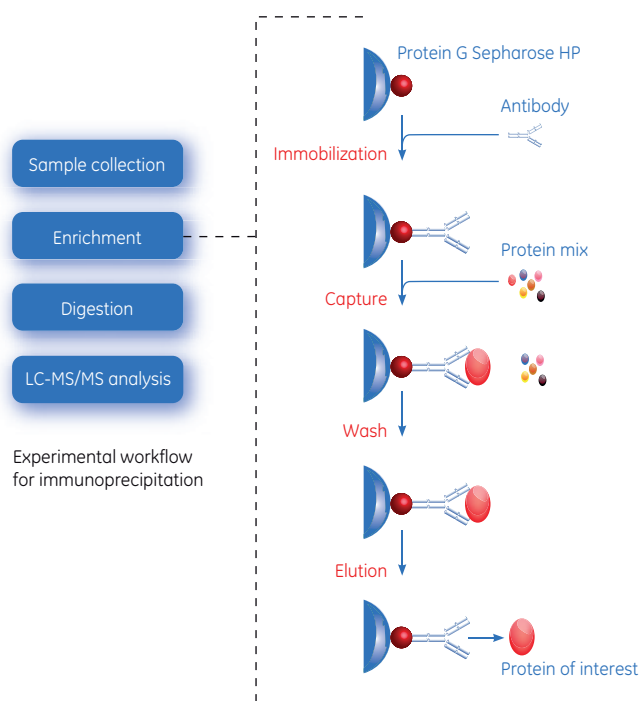
Elution buffer: 0.1 M glycine with 2 M urea, pH 3.0

High-throughput method: Manual centrifugation

Sample preparation of eluted sample prior to analysis:

Cysteines were reduced and alkylated, and peptides were generated by trypsin cleavage.

Analysis method: MS on Ettan MDLC system coupled to a LTQ linear ion trap from Thermo Scientific



Results from LC-MS/MS analysis. The number of unique proteins identified in each sample is shown, with start material in green and hTf enriched sample in blue. hTf was only found in the enriched sample.

Forty-eight peptides of human serotransferrin were identified giving a sequence coverage of 70% as shown by the red boxes compared to the theoretical sequence (black line). An example of an MS/MS spectrum is shown.

Summary

The target molecule, which could not be detected in the original sample, was easily identified by LC/MS analysis after enrichment on Protein G HP MultiTrap.

Protein enrichment using Streptavidin HP MultiTrap

When the antibody for a target protein has poor affinity for Protein A and Protein G, a biotinylated antibody and Streptavidin HP MultiTrap can be used. To demonstrate this, human serum albumin (HSA) was added to *E. coli* lysate containing human serum albumin (HSA) at a concentration of 0.15% of the total *E. coli* protein content, approximately corresponding to the concentration of a medium-abundant protein. The standard protocol for manual centrifugation was used.

Capture of HSA was achieved by first immobilizing a biotinylated antibody (polyclonal rabbit anti-human albumin) to Streptavidin HP MultiTrap, after which the spiked protein sample was added.

Analysis of the collected fractions by SDS-PAGE showed significant enrichment of HSA. The target protein was enriched 180-fold relative to the starting material.

96-well plate: Streptavidin HP MultiTrap

Sample: 200 μ l/well of clarified *E. coli* extract containing 7.5 μ g/ml human serum albumin, total protein conc. 5 mg/ml

Antibody: Polyclonal rabbit anti-human albumin (biotinylated)

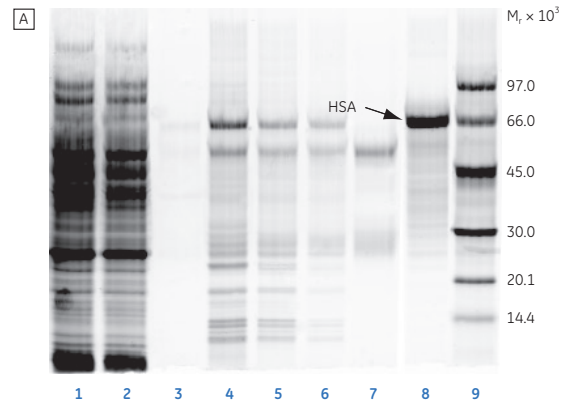
Binding buffer: 50 mM Tris, 150 mM NaCl, pH 7.5

Wash buffer: 50 mM Tris, 150 mM NaCl, 2 M urea, pH 7.5

Elution buffer: 0.1 M glycine with 2 M urea, pH 3.0

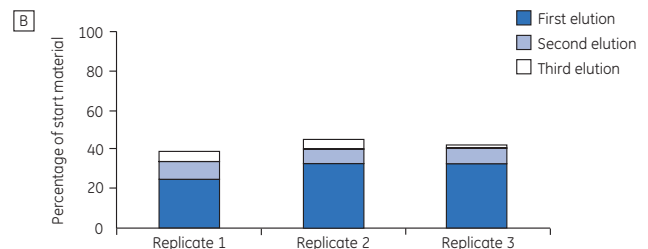
High-throughput method: Manual centrifugation

Analysis method: SDS-PAGE analysis, gel post-stained with Deep Purple Total Protein Stain and scanned using Ettan DIGE Imager



Lanes

1. Start material (diluted 1:30)
2. Flowthrough (diluted 1:30)
3. Fifth wash
4. First elution
5. Second elution
6. Third elution
7. Antibody
8. HSA, M, 65 000
9. LMW markers



Enrichment of HSA from *E. coli* cell lysate. **(A)** Analysis by SDS-PAGE (wash steps 1 to 4 have been omitted from the gel). The gel was post-stained with Deep Purple Total Protein Stain and scanned using Ettan DIGE Imager. **(B)** All three elution steps were analyzed using ImageQuant TL software.

Summary

The target protein is enriched approximately 180-fold relative to the starting material, enabling simpler and more reliable analysis and quantitation.

Optimization of desalting/buffer exchange

Desalting of protein samples is often required in order to perform subsequent analyses such as mass spectrometry and for label-free study of protein interactions using Biacore™ systems. The PD MultiTrap G-25 gives highly reproducible and efficient desalting with high levels of protein recovery, typically over 85%. In the example below, removal of NaCl from BSA was 93% and well-to-well variation was 1% relative standard deviation.

Desalting at three different protein concentrations (0.25, 0.5, or 1 mg BSA/ml) and three different salt concentrations (0.25, 0.5, or 1 M NaCl) showed identical desalting behavior (all data not shown). The levels of protein recovery and desalting are dependent on the sample volume, with high salt removal at low volumes, and high protein recovery at high volumes. Therefore, sample volumes can be used as a way to regulate high desalting or high recovery, depending on the requirements of your analysis.

Highly reproducible desalting

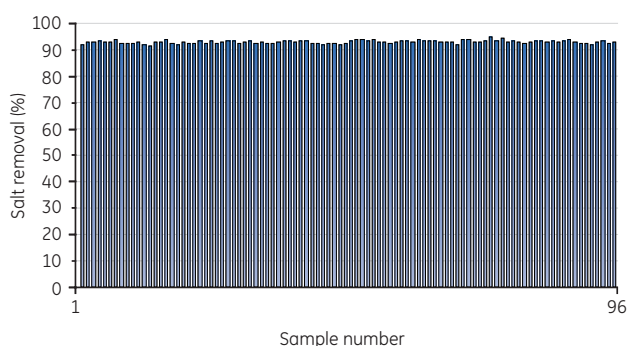
96-well plate: PD MultiTrap G-25

Sample: 130–1000 µl bovine serum albumin in 1 M NaCl

Equilibration buffer: Milli-Q™ water

High-throughput method: Manual centrifugation

Detection method: Conductivity measurement



Optimization of volume for protein recovery and desalting

96-well plate: PD MultiTrap G-25

Samples: 1) Tris-HCl, pH 7 + 1 M NaCl + 1 mg/ml BSA

2) Tris-HCl, pH 7 + 0.5 M NaCl + 0.5 mg/ml BSA

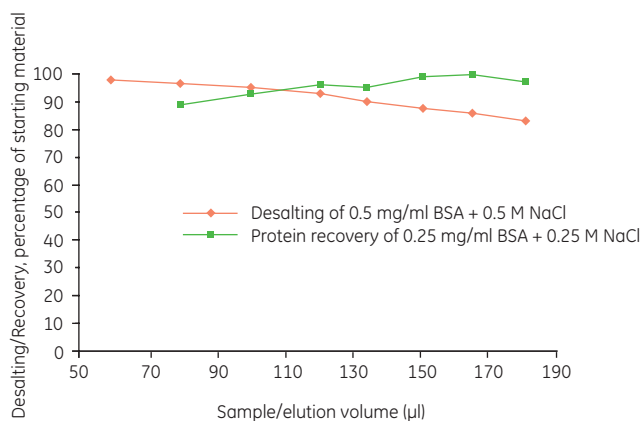
3) Tris-HCl, pH 7 + 0.25 M NaCl + 0.25 mg/ml BSA

Sample volumes: 60, 80, 100, 120, 135, 150, 165, and 180 µl

Equilibration buffer: 20 mM Tris-HCl, pH 7

High-throughput method: Manual centrifugation, Beckman Coulter Avant J-26 XP

Detection method: Absorbance at 280 nm for recovery and conductivity for salt content



Summary

PD MultiTrap G-25 desalts many samples simultaneously and uniformly. The volume of sample can be optimized to obtain either high desalting capacity or high protein recovery.

How to get the best from MultiTrap

General advice

- To reduce the risk of cross-contamination, dilute viscous samples first. This helps to avoid foaming and droplet formation under the filter plates.
- When using the affinity-based systems, especially Protein A HP MultiTrap and Protein G HP MultiTrap, shake the plates for 10 min after sample loading.
- Avoid applying more than 500 µg of target protein per well.
- To avoid contamination, cover MultiTrap and collection plates with Microplate Foil during manual shaking or centrifugation.
- When you are working with very low volumes or amounts, we recommend an incubation time of at least 1 min between addition of the dispensing elution buffer and evacuation (by vacuum or centrifugation).
- MultiTrap can be eluted by either centrifugation or vacuum. Centrifugation gives more reproducible results, but vacuum elution involves less hands-on time.

Vacuum protocol guidelines

- When using a liquid handling station or a robot, the pipettes should be washed with an appropriate buffer repeatedly between different samples. Check the instrument's recommendations. Alternatively, use disposable tips.
- After vacuum elution, spin the collection plate at 800 × g for 2 min to remove bubbles prior to analysis.
- After sample loading, use three washing steps to remove unbound material.
- Keep the space between MultiTrap and the collection plate less than 5 mm.
- After the last washing step, the plate should be pressed gently against a filter paper to remove any liquid attached under the plate. This step can be programmed on a robotic system.
- Setting the correct elution time is important to avoid splashing. Use a pressure drop of 160 mbar for 45 to 60 s to evacuate protein from wells, followed by 300 mbar for 3 s.
- Use deep-well collection plates (≥ 500 µl/well) to reduce the risk of splashing.

Centrifugation protocol guidelines

- MultiTrap is compatible with most centrifuges, and is compatible with most collection plates. To find out if your centrifuge is suitable, check the space in the centrifuge rotor compartment. Select a MultiTrap and collection plate combination that fits the available depth. Most often, both deep-well plates as well as shallow plates can be used.

Protocols for affinity techniques using MultiTrap

MultiTrap takes up to 600 µl of sample in each well. This yields up to 0.5 mg of the target protein. The protocols are similar for all affinity-based products.

The incubation time often needs to be optimized for the specific sample.



Vacuum

- Place MultiTrap on collection plate. Apply vacuum at -300 mbar for 30 s
- Apply vacuum at -300 mbar for 30 s
- Apply vacuum at -300 mbar for 30 s Repeat once
- Incubate with shaking for 3-10 min. Apply vacuum at -160 mbar for 40 sec, -300 mbar for 3 s
- Apply vacuum at -300 mbar for 45-120 s Repeat twice. After last wash, remove any droplets under the filter plate by pressing it against a filter paper
- Place a new collection plate under the filter plate before elution of the sample
- Mix for 1 min. Apply vacuum at -160 mbar for 45 s to 1 min followed by -300 mbar for 3 s Repeat twice. If elution is incomplete, increase time

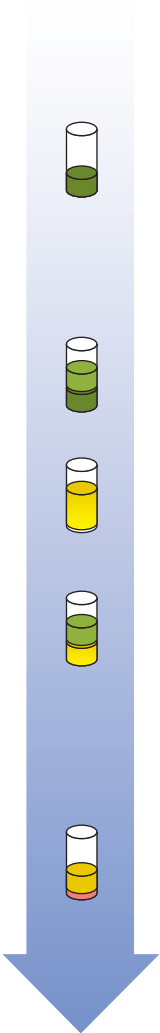
Step

- Enzymatic/chemical/mechanical lysis of cells 10-40 min
- Removing storage solution
- Washing wells with distilled water
- Equilibrating the media with binding buffer
- Loading of unclarified sample or clarified lysate
- Washing bound material with binding buffer
- Eluting the resulting protein with the appropriate elution buffer.



Centrifugation

- Place MultiTrap on collection plate. Centrifuge at 500 x g for 2 min
- Centrifuge at 500 x g for 2 min
- Centrifuge at 500 x g for 2 min. Repeat once
- Incubate for 3-10 min. with shaking Centrifuge at 100 x g for 2 min
- Centrifuge at 500 x g for 2 min. Repeat three times
- Place a new collection plate under the filter plate before elution of the sample
- Mix for 1 min. Centrifuge fractions at 500 x g for 2 min and collect fraction. Repeat once



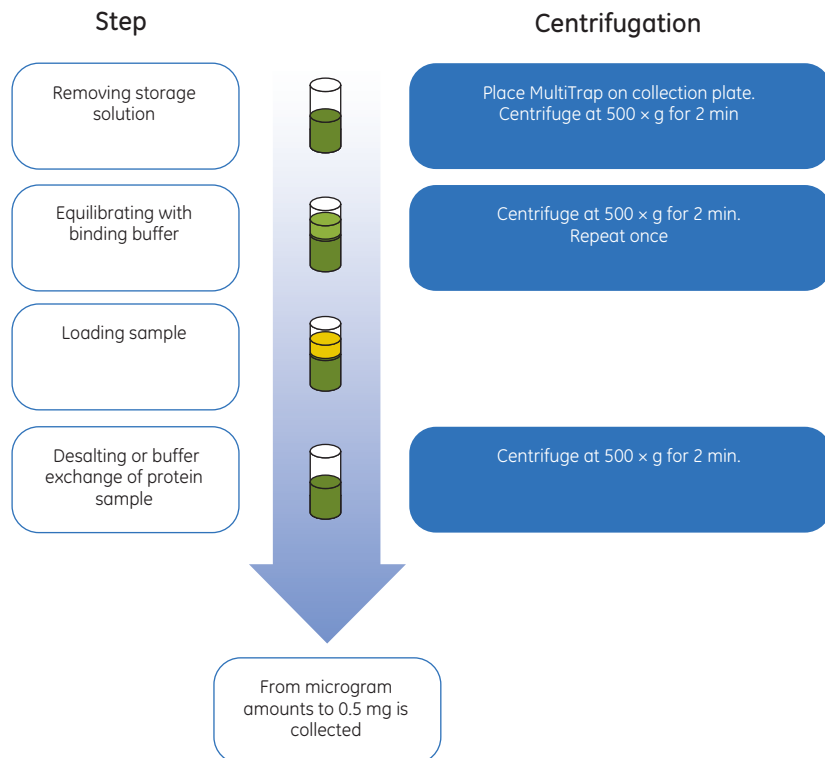
From microgram amounts to 0.5 mg is collected

More detailed protocols for centrifugation and vacuum can be found in the instructions accompanying each product.

Protocol for desalting/buffer exchange using PD MultiTrap G-25

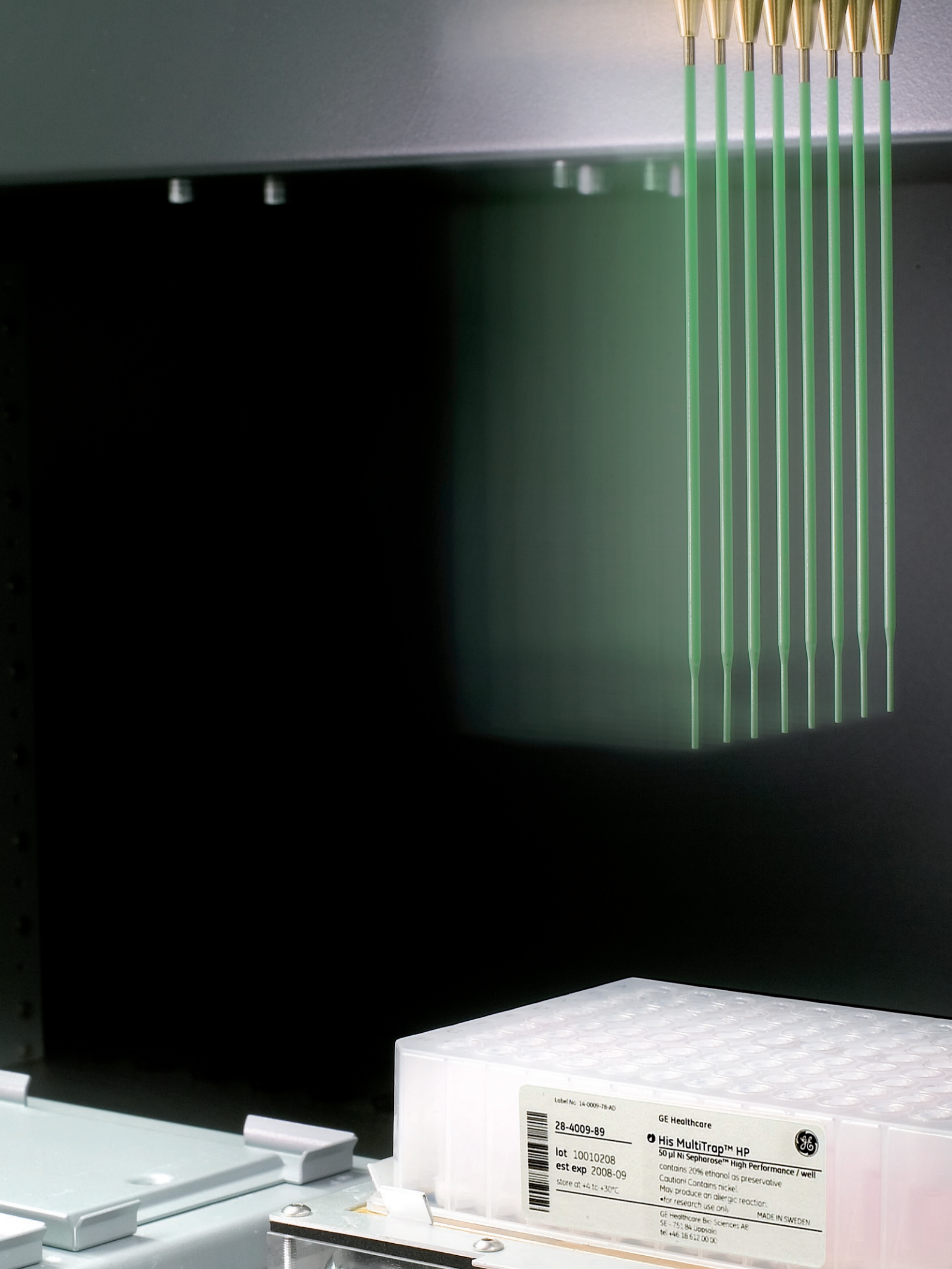
Desalting and buffer exchange with PD MultiTrap G-25 is based on gel filtration. This product should only be used with a centrifugation protocol and with a recommended sample

volume of 110 to 130 μ l for optimal protein recovery and desalting effect.



MultiTrap products

Product & medium	Mode of action	Target molecule	Applications	Quantity	Code No.
His MultiTrap FF Ni Sepharose™ 6 Fast Flow	IMAC - immobilized metal ion affinity chromatography	Histidine-tagged proteins	Screening, small-scale purification	4 × 96-well plates	28-4009-90
His MultiTrap HP Ni Sepharose High Performance	IMAC - immobilized metal ion affinity chromatography	Histidine-tagged proteins	Screening, small-scale purification	4 × 96-well plates	28-4009-89
GST MultiTrap FF Glutathione Sepharose 4 Fast Flow	Affinity chromatography	GST-tagged proteins	Screening, small-scale purification	4 × 96-well plates	28-4055-01
GST MultiTrap 4B Glutathione Sepharose 4B	Affinity chromatography	GST-tagged proteins	Screening, small-scale purification	4 × 96-well plates	28-4055-00
Streptavidin HP MultiTrap Streptavidin Sepharose High Performance	Affinity chromatography	Biotinylated biomolecules	Protein enrichment, protein-protein interaction investigations	4 × 96-well plates	28-9031-31
Protein A HP MultiTrap Protein A Sepharose High Performance	Affinity chromatography	Fc region of IgG	Protein enrichment, small-scale purification	4 × 96-well plates	28-9031-33
Protein G HP MultiTrap Protein G Sepharose High Performance	Affinity chromatography	Fc region of immunoglobulins	Protein enrichment, small-scale purification	4 × 96-well plates	28-9031-35
PD MultiTrap G-25 Sephadex™ G-25 Medium	Gel filtration		Desalting and buffer exchange	4 × 96-well plates	28-9180-06
Collection plate				5 × 96-well plates	28-4039-43
Microplate Foil				100 foils	Biacore BR-1005-78
UNIFILTER™ Lysate clarification 1 800 µl			Lysate clarification	1 × 96-well plates	Whatman 7720-2830
UniVac™ 3 100 µl to 10 ml			Vacuum to collect manifold	1 manifold	Whatman 7705-0106



Label No. 14-0009-78-A0

28-4009-89

lot 10010208
est exp 2008-09
store at: +4 to +30°C

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His MultiTrap™ HP
50 µl Ni Sepharose™ High Performance / well
contains 20% ethanol as preservative
Caution! Contains nickel!
May produce an allergic reaction.
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tel: +46 18 612 00 00

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please visit, www.gelifesciences.com/contact

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GST Gene Fusion Vectors: A license for commercial use of GST Gene Fusion Vectors under US patent 5,654,176 and equivalent patents and patent applications in other countries must be obtained from Millipore Corp (formerly Chemicon International Inc).

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