Streptavidin HP SpinTrap
Streptavidin HP MultiTrap
Streptavidin HP SpinTrap Buffer Kit

Streptavidin HP SpinTrap™ and Streptavidin HP MultiTrap™ (Fig 1) are prepacked, single-use spin columns and 96-well filter plates for the preparation of protein samples and enrichment of proteins of interest from clarified cell lysates and biological fluids. These spin columns and 96-well filter plates address the need for flexible, small-scale preparation of protein samples before downstream analyses such as gel electrophoresis, liquid chromatography, and LC-MS. The Streptavidin HP SpinTrap Buffer Kit can be used for increased convenience and reproducibility between runs.

The key benefits of Streptavidin HP SpinTrap and Streptavidin HP MultiTrap are:

- Reproducible capture performance, run for run; required for quantitative and comparative expression studies.
- Yield; each toolkit includes an Optimization Guide to maximize recovery of your protein of interest.
- Optimized for downstream analysis; protocols are designed and tested for several different analyses, for example electrophoresis and LC-MS.
- Purity; protocols support performance optimization, modification, and troubleshooting.

Streptavidin HP SpinTrap and Streptavidin HP MultiTrap utilize the strong interaction between biotin and streptavidin, which is immobilized on the prepacked Streptavidin Sepharose™ High Performance medium. The Streptavidin HP SpinTrap Buffer Kit is designed for small-scale affinity preparation and protein enrichment/immunoprecipitation

Fig 1. Streptavidin HP SpinTrap columns and MultiTrap 96-well filter plates are designed for efficient, small-scale enrichment of proteins of interest from clarified cell lysates and biological fluids and can be used with the Streptavidin HP SpinTrap Buffer Kit for increased convenience.

using the Streptavidin HP SpinTrap columns and contains reagents sufficient for 16 reactions. The kit consists of both stock solutions and reagents that are ready for use. Working solutions are prepared by adding distilled water directly to the stock solution bottle. The kit eliminates time-consuming buffer preparation and thus promotes fast, reproducible and convenient enrichment of a target protein from a complex protein sample.

Biotinylated affinity ligands (antibodies, proteins, or aptamers) immobilize tightly to the medium and the protein of interest can be enriched separately up to several hundred-fold, depending on the ligand used. Runs are performed in parallel, which ensures fast and reliable capture of proteins of interest from a large number of complex samples. The products also enable study of protein–protein interactions where protein complexes are to be analyzed.

Table 1. Characteristics of prepacked Streptavidin HP SpinTrap columns, Streptavidin HP MultiTrap 96-well filter plates and Streptavidin HP SpinTrap Buffer Kit.

Prepacked medium	Streptavidin Sepharose High Performance
Matrix	Highly cross-linked agarose, 6%
Ligand	Streptavidin
Ligand coupling method	N-hydroxysuccinimide activation
Ligand density	10 µmol streptavidin/ml medium
Binding capacity[1]	> 300 biotin nmol/ml medium 6 mg biotinylated BSA/ml medium
Average particle size	34 µm
pH stability[2]	4–9 (long term), 2–10.5 (short term)
Working temperature	4°C to 30°C
Storage solution	20% ethanol
Storage temperature	4°C to 8°C
Streptavidin HP SpinTrap	
Volume of prepacked medium	100 µl
Column volume	800 µl
Column material	Polypropylene and polyethylene
Streptavidin HP MultiTrap	
Filter plate size[3]	127.8 × 85.5 × 30.6 mm
Prepacked medium volume/well	50 µl
Well volume	800 µl
Filter plate material	Polypropylene and polyethylene
Centrifugation speed[4]	700 × g
Vacuum pressure[4]	
Recommended	–0.1 to –0.3 bar
Maximum	–0.5 bar

[1] Protein dependent

[2] pH below 3 is sometimes required to elute strongly bound antibody species. However, protein ligands may hydrolyze at very low pH

[3] According to American National Standards Institute (ANSI) and Society for Biomolecular Screening (SBS) standards 1-2004, 3-2004, and 4-2004

[4] Actual settings depend on the sample properties and pretreatment

Streptavidin HP SpinTrap Buffer Kit

Buffer	Content	Formulation	Volume
Binding/Washing Buffer (TBS)	0.5 M Tris; 1.5 M NaCl, pH 7.5	10x	2x5 ml
Elution Buffer	1 M Glycine-HCl, pH 2.9	10x	3 ml
Blocking Buffer	2 mM Biotin; 50 mM Tris; 0,15 M NaCl, pH 7.5	Ready to use	20 ml

Characteristics

Streptavidin HP SpinTrap and Streptavidin HP MultiTrap are both prepacked with Streptavidin Sepharose High Performance, a proven medium with high affinity for biotin. Reliable sample preparation is achieved through the fast kinetics and high binding capacity of the medium. The agarose-based medium provides a hydrophilic and chemically favorable environment for coupling, while the highly cross-linked structure of the 34-µm spherical beads ensures excellent flow of sample through the spin columns and the 96-well filter plates. The main characteristics are listed in Table 1.

Protocol optimization and operation

Major advantages of Trap products are the flexibility and reproducibility of the protocols, and full descriptions of the components of each product. In addition, Streptavidin HP SpinTrap and Streptavidin HP MultiTrap are easy to use. SpinTrap columns only require a standard microcentrifuge. MultiTrap 96-well filter plates allow sample preparation by centrifugation or vacuum, either operated manually or automated using robotics.

Each toolkit includes suggestions for protocol optimization to maximize the recovery of your protein of interest; see Instructions 28-9067-74 (Streptavidin HP SpinTrap) and 28-9067-75 (Streptavidin HP MultiTrap). Elution can be performed using the buffers described in Table 2.

Table 2. Suggested elution buffers for various situations

Glycine/HCl, 1–2 M urea, pH 2.5–3.1	Most antibody-antigen bonds are broken and sufficient elution is usually achieved. This is often a first-choice buffer to screen for optimal elution conditions.
Glycine/HCl, pH 2.5–3.1	Many antibody-antigen bonds are broken and sufficient elution is usually achieved.
0.5 M acetic acid	Low pH buffer compatible with mass spectrometry due to the volatility of acetic acid.
2% SDS	Breaks all protein-protein bonds and solubilizes even the most difficult proteins. Can be used in aqueous solution or as an additive to other buffers. SDS is often a constituent of electrophoresis loading buffer making it compatible with many electrophoresis procedures.
Citric acid, pH 2.5-3.1	Many antibody-antigen bonds are broken and sufficient elution is usually achieved.
0.1 M ammonium hydroxide	A basic elution buffer used, for instance, when the protein of interest is acid labile.

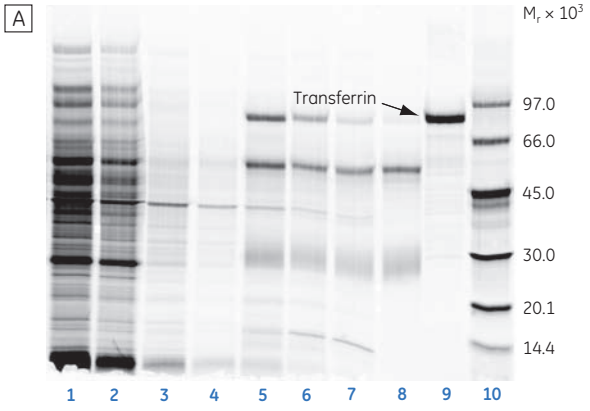
Flexible protocols enable reliable protein enrichment/immunoprecipitation

Enrichment of a particular protein is often desired to increase its signal in subsequent analysis steps. To demonstrate the efficiency and reproducibility of the protocols for Streptavidin HP SpinTrap and Streptavidin HP MultiTrap, human transferrin and human serum albumin (HSA) were enriched after being added to an *E. coli* protein sample. The concentration of the protein of interest was 0.15% of the total *E. coli* protein content, which approximately corresponds to the concentration of a medium-abundant protein. Capture of the protein of interest was achieved using a biotinylated antibody (polyclonal rabbit anti-human transferrin or polyclonal rabbit anti-human albumin) that was immobilized on the medium.

Analysis by SDS-PAGE of the collected fractions from the runs revealed a significant enrichment of transferrin and HSA (Fig 2A and 3A, respectively). Recovery of the start material was 60% to 70% for SpinTrap and around 40% for MultiTrap with the majority of the protein eluted in the first elution step (Fig 2B and 3B). With the target protein being 0.15% of the total *E. coli* protein content, the enrichment of the protein of interest relative to the start material was approximately 100-fold with Streptavidin HP SpinTrap and 180-fold with Streptavidin HP MultiTrap.[1] Quantitation of eluted protein of interest was performed using standard curves with known amounts of transferrin and HSA (data not shown).

[1] Minor leakage of antibody from the medium may be seen. This is antibody concentration dependent and can be minimized by optimizing the amount of coupled antibody with regard to the antigen concentration.

Trap product: Streptavidin HP SpinTrap
Sample: 5 mg/ml *E. coli* protein containing 7.5 µg/ml human transferrin
Sample volume: 200 µl
Antibody: Polyclonal rabbit anti-human transferrin (biotinylated)
Binding buffer: Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer: TBS, 2 M urea, pH 7.5
Elution buffer: 0.1 M glycine/HCl, 2 M urea, pH 3.0



Lanes
1. Flowthrough (diluted 1:30)
2. First wash (diluted 1:10)
3. Third wash
4. Fifth wash
5. First elution
6. Second elution
7. Third elution
8. Antibody
9. Transferrin, M_r 77 000
10. LMW markers

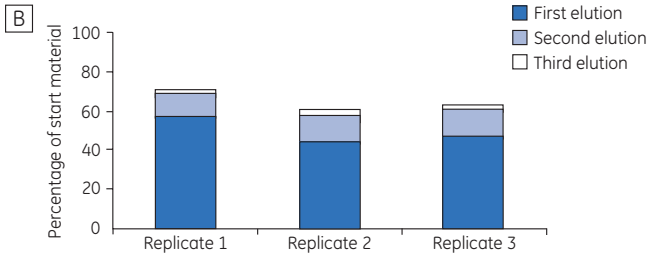
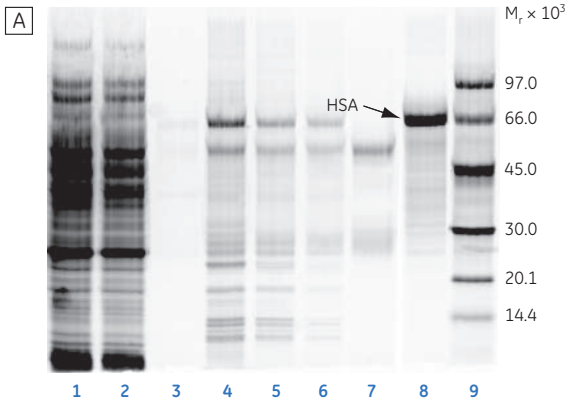


Fig 2. Enrichment of transferrin from *E. coli* cell lysate. **(A)** Analysis by SDS-PAGE (wash steps 2 and 4 have been omitted from the gel). The gel was poststained with Deep Purple™ Total Protein Stain and scanned using Ettan™ DIGE Imager. **(B)** All three elution steps were analyzed using ImageQuant™ TL software. Recovery (percentage of start material) of three replicates is shown.

Trap product: Streptavidin HP MultiTrap
Sample: 5 mg/ml *E. coli* protein containing 7.5 µg/ml human serum albumin (HSA)
Sample volume: 200 µl
Antibody: Polyclonal rabbit anti-human albumin (biotinylated)
Binding buffer: Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer: TBS, 2 M urea, pH 7.5
Elution buffer: 0.1 M glycine/HCl, 2 M urea, pH 3.0



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_r 65 000
9. LMW markers

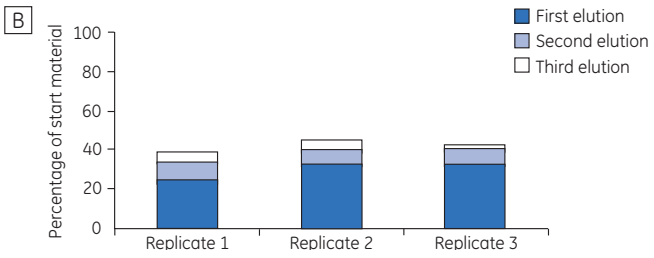


Fig 3. Enrichment of HSA from *E. coli* cell lysate. **(A)** Analysis by SDS-PAGE (wash steps 1 to 4 have been omitted from the gel). The gel was poststained with Deep Purple Total Protein Stain and scanned using Ettan DIGE Imager. **(B)** All three elution steps were analyzed using ImageQuant TL software. Recovery (percentage of start material) of three replicates is shown.

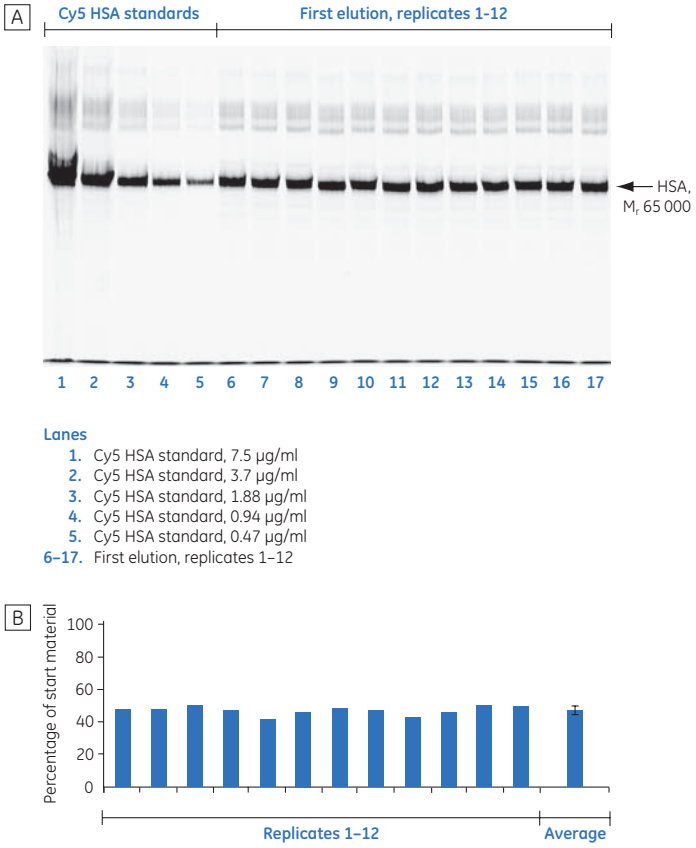
Reproducible capture performance

To correlate protein expression with, for example, disease or treatment, large numbers of samples must be prepared and analyzed. This is tedious work and a source of error. SpinTrap columns and MultiTrap 96-well filter plates offer reproducibility, flexibility, and convenience, thus minimizing variation during the enrichment step.

In a run of 12 parallel Streptavidin HP SpinTrap columns with *E. coli* protein containing HSA, the recovery of total loaded material varied by 6% (relative standard deviation, Fig 4) and the purity varied by 13%.

A similar experiment was performed using the MultiTrap format but with Protein A Sepharose High Performance medium (Protein A HP MultiTrap). In this case the well-to-well variation was below 10% (relative standard deviation) for both purity and recovery (see Data File 28-9067-89 AB, Protein A HP SpinTrap and Protein A HP MultiTrap).

Trap product: Streptavidin HP SpinTrap
Sample: 5 mg/ml *E. coli* protein containing 7.5 µg/ml human serum albumin (HSA)
Sample volume: 200 µl
Antibody: Polyclonal rabbit anti-human albumin (biotinylated)
Binding buffer: Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer: TBS, 2 M urea, pH 7.5
Elution buffer: 0.1 M glycine/HCl, 2 M urea, pH 3.0



Lanes
1. Cy5 HSA standard, 7.5 µg/ml
2. Cy5 HSA standard, 3.7 µg/ml
3. Cy5 HSA standard, 1.88 µg/ml
4. Cy5 HSA standard, 0.94 µg/ml
5. Cy5 HSA standard, 0.47 µg/ml

6–17. First elution, replicates 1–12

Fig 4. Enrichment of HSA from *E. coli* lysate. For visualization purposes, HSA was labeled with CyDye™ DIGE Fluor Cy5™ minimal dye. Twelve columns were run in parallel. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager. Analysis was performed using ImageQuant TL software. **(A)** The first elution step for each Cy5 labeled sample is shown. Known amounts of HSA were run as standards (lanes 1 to 5). **(B)** Recovery of total loaded material varied by 6% (relative standard deviation), illustrated by the error bar on the column showing the average of the 12 replicates.

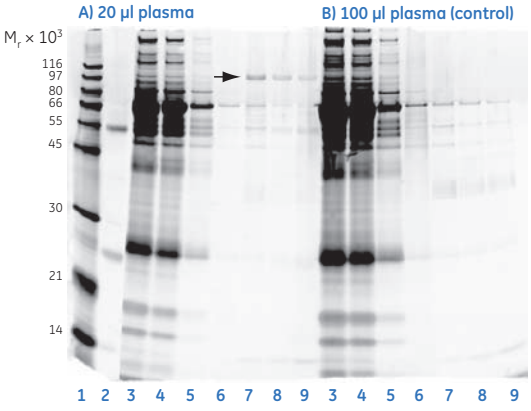
Application

Enrichment of plasminogen from human plasma

To show the performance of Streptavidin HP SpinTrap, plasminogen was enriched from human plasma using a biotinylated monoclonal antibody. The concentration of the total protein was around 50 mg/ml and the concentration of the plasminogen was between 0.2 and 0.3 mg/ml, which is equivalent to 0.5% of the total protein concentration.

Figure 5 shows the SDS-PAGE result of enrichment of plasminogen using Streptavidin HP SpinTrap.

Trap product: Streptavidin HP SpinTrap
Sample: Human plasma
Sample volumes: 20 and 100 µl
Antibody: Monoclonal mouse anti-plasminogen (biotinylated)
Binding buffer: Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer: TBS, 2 M urea, pH 7.5
Elution buffer: 0.1 M glycine/HCl, 2 M urea, pH 3.0



Lanes
1. Protein Molecular Weight Standards (broad range)
2. Antibody
3. Flowthrough (diluted 1:30)
4. First wash (diluted 1:10)
5. Third wash
6. Fifth wash
7. First elution
8. Second elution
9. Third elution

Fig 5. Enrichment of plasminogen from human plasma. **(A)** Analysis by SDS-PAGE (wash steps 2 and 4 have been omitted from the gel). The gel was poststained with Deep Purple Total Protein Stain and scanned using Ettan DIGE Imager. The arrow indicates the position of the plasminogen (M_r 93 000). **(B)** The control sample was run in an identical manner compared with the plasma sample, but without a coupled antibody.

Ordering information

Products	Quantity	Code no.
Streptavidin HP SpinTrap	16 columns	28-9031-30
Streptavidin HP SpinTrap Buffer Kit	1	28-9135-68
Streptavidin HP MultiTrap	4 × 96-well plates	28-9031-31
Collection plate 500 µl V-bottom (for collection of fractions from MultiTrap)	5 × 96-well plates	28-4039-43
Related products		
Sample Grinding Kit	50 samples	80-6483-37
Protease Inhibitor Mix	1 ml	80-6501-23
Nuclease Mix	0.5 ml	80-6501-42
Protein A HP SpinTrap	16 columns	28-9031-32
Protein A HP MultiTrap	4 × 96-well plates	28-9031-33
Protein G HP SpinTrap	16 columns	28-9031-34
Protein G HP MultiTrap	4 × 96-well plates	28-9031-35
NHS HP SpinTrap	5 ml medium and 24 empty spin columns	28-9031-28
Ab SpinTrap	50 × 100 µl	28-4083-47
Ab Buffer Kit	1	28-9030-59

Literature

Antibody purification Handbook	18-1037-46
Affinity Chromatography Handbook	18-1022-29
Affinity Chromatography Columns and Media Selection Guide	18-1121-86

For contact information for your local office, please visit, www.gelifesciences.com/contact

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