Protein G HP SpinTrap
Protein G HP MultiTrap
Protein A/G SpinTrap Buffer Kit

Protein G HP SpinTrap™ and Protein G HP MultiTrap™ (Fig 1) are designed for the enrichment of proteins of interest, immunoprecipitation, and the purification of monoclonal and polyclonal antibodies from cell lysates and biological fluids. Protein G HP SpinTrap are prepacked, single-use spin columns. Protein G HP MultiTrap are prepacked 96-well filter plates. Both products contain Protein G Sepharose™ High Performance.

The spin columns and 96-well filter plates address the need for flexible, small-scale preparation of protein samples before downstream protein analyses such as gel electrophoresis, liquid chromatography, and LC-MS. When using the columns and plates for protein enrichment, an antibody of choice is first immobilized onto the Protein G media, and then used for the capture and enrichment of the protein of interest. The protein can be enriched several hundredfold, depending on the specificity of the antibody.

The benefits of Protein G HP SpinTrap and Protein G HP MultiTrap when running the protein enrichment/ immunoprecipitation protocols are:

- Reproducible performance from run to run enables quantitative and comparative expression studies
- High performance for yield and purity, which can be further increased using the supplied Optimization Guide that supports performance optimization, protocol modifications, and troubleshooting.
- Protocols that have been tested and optimized for downstream analyses such as electrophoresis and LC-MS.

Fig 1. Protein G 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 Protein A/G HP SpinTrap Buffer Kit for increased convenience. They can also be used for antibody purification

- Choice of protocols; a classic protocol for speed or a cross-link protocol ensuring elution of the protein of interest only, with the antibody remaining bound to the column.

For antibody purification, the benefits of Protein G HP SpinTrap and Protein G HP MultiTrap are:

- High purity and yield of antibodies.
- Simple and proven methods for reproducible results.
- Quick purification due to the prepacked format and the absence of pretreatment.

Protein G HP SpinTrap and the Ab SpinTrap are identical columns. The difference between the products is the pack size (16 and 50 columns respectively). Both products include the same protocols: purification of antibodies as well as enrichment of a target protein. For antibody purification applications, please see the Ab SpinTrap and Ab Buffer Kit Data File: 28-9020-30. For simplification, the column is referred to as Protein G HP SpinTrap throughout this document.

Buffer Kit

Protein A/G SpinTrap Buffer Kit (Fig 1) is designed for protein enrichment using Protein A HP or Protein G HP SpinTrap columns. The kit is sufficient for 16 reactions and contains reagents for both the Classic and Cross-link protocols (with or without elution of antibody together with target protein). Some of the reagents are delivered in two bottles for increased stability. The kit consists of both stock solutions and reagents ready for use. Working solutions are prepared by adding distilled water directly into the stock solution bottle.

The kit eliminates time-consuming buffer preparation and thus promotes fast, reproducible and convenient enrichment of target protein from a complex protein sample.

Characteristics

Protein G HP SpinTrap and Protein G HP MultiTrap are both prepacked with Protein G Sepharose High Performance, a proven medium with strong affinity for IgG subclasses. Reliable sample preparation is achieved through fast kinetics and high binding capacity of the medium. Specificity for capture of the protein is achieved by immobilization of antibodies to the Sepharose beads through well-established coupling techniques (Fig 3). 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. Table 1 lists the main characteristics of the products.

Table 1. Characteristics of prepacked Protein G HP SpinTrap columns, Protein G HP MultiTrap 96-well filter plates, and Protein A/G SpinTrap Buffer Kit.

Prepacked medium	Protein G Sepharose High Performance
Matrix	Highly cross-linked agarose, 6%
Ligand	Recombinant protein G lacking albumin-binding region
Ligand coupling method	N-hydroxysuccinimide activation
Ligand density	Approx. 2 mg protein G/ml medium
Binding capacity[1]	> 10 mg human IgG/ml medium
Average particle size	34 µm
pH stability[2]	3–9 (long term), 2–9 (short term)
Working temperature	4°C to 30°C
Storage solution	20% ethanol
Storage temperature	4°C to 30°C
Protein G HP SpinTrap	
Volume of prepacked medium	100 µl
Column volume	800 µl
Column material	Polypropylene and polyethylene
Protein G 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	
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

Protein A/G SpinTrap Buffer Kit

Buffer	Content	Formulation	Volume
Binding/Washing Buffer (TBS)	0.5 M Tris; 1.5 M NaCl, pH 7.5	10×	2×5 ml
Elution Buffer (Classic)	2.5 % HAc	Ready to use	20 ml
Elution Buffer (Cross-link)	1 M Glycine-HCl, pH 2.9	10×	3 ml
Cross-link Solution A	2 M Triethanolamine, pH 8.9	10×	4 ml
Cross-link Solution B	1 M Ethanolamine, pH 8.9	10×	2×1 ml

Antibody purification

The purification of antibodies with Protein G HP SpinTrap/Ab SpinTrap or Protein G HP MultiTrap can be divided into four stages; equilibration, sample incubation, wash, and elution (Fig 2). Each step involves centrifugation.

Binding of the antibody is performed at neutral pH, and elution by lowering the pH. Eluted material is collected in tubes containing neutralizing buffer to preserve the activity of acid-labile IgG's.

Purifying antibodies with Protein G HP SpinTrap/Ab SpinTrap or Protein G HP MultiTrap is a simple, four-stage procedure that can be completed in less than 20 min: (1) Equilibrate by adding binding buffer and centrifuge; (2) add sample and incubate; (3) wash with binding buffer; (4) elute the target protein with elution buffer.

Recommended buffers:

Binding buffer: 20 mM sodium phosphate, pH 7.0

Elution buffer: 0.1 M glycine-HCl, pH 2.7

Neutralizing buffer: 1 M Tris-HCl, pH 9.0

Ab Buffer Kit is available as an accessory for increased convenience (Fig 1). The kit contains buffer solutions for Binding, Elution and Neutralizing optimized for rapid purification of monoclonal and polyclonal IgG with immobilized Protein A or Protein G media. The kit eliminates time-consuming buffer preparation and thus promotes fast, reproducible and convenient purification work.

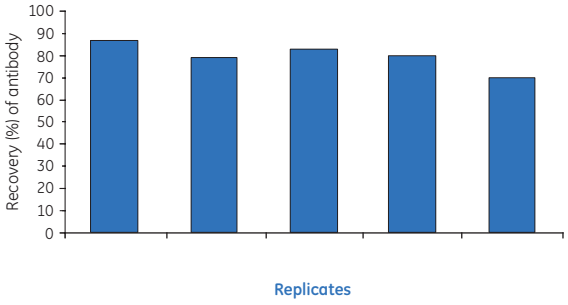


Fig 2. Reproducibility of recovery of human IgG (Gammanorm) by Protein G HP MultiTrap, 0.5 mg loaded/well, performed with the Ab purification protocol using buffer volumes adjusted for 50 µl gel/well.

Table 2. Relative binding strengths of antibodies from various species to protein G as measured in a competitive ELISA test. Antibody binding strength to protein A is also shown for comparison. GE Healthcare also offers prepacked Protein A HP SpinTrap and Protein A HP MultiTrap; see Ordering information for details

Species	Subclass	Protein G binding	Protein A binding
Human	IgA	—	variable
	IgD	—	—
	IgE	—	—
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	++++	—
	IgG_4	++++	++++
	IgM*	—	variable
Avian egg yolk	IgY†	—	—
Cow		++++	++
Dog		+	++
Goat		++	—
Guinea pig	IgG_1	++	++++
	IgG_2	++	++++
Hamster		++	+
Horse		++++	++
Koala		+	—
Llama		+	—
Monkey (rhesus)		++++	++++
Mouse	IgG_1	++++	+
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	+++	++
	IgM*	—	variable
Pig		+++	+++
Rabbit		+++	++++
Rat	IgG_1	+	—
	IgG_{2a}	++++	—
	IgG_{2b}	++	—
	IgG_3	++	+
Sheep		++	+/—

* Purified using HiTrap™ IgM Purification HP columns, code no. 17-5110-01

† Purified using HiTrap IgY Purification HP columns, code no. 17-5111-01

++++ = strong binding
++ = medium binding
— = weak or no binding

Reproducible protein enrichment/immunoprecipitation

Enrichment of a particular protein is often desired in order to increase its signal in subsequent analysis steps. Protein G HP SpinTrap and MultiTrap are used to immobilize a biospecific ligand (i.e., an antibody) with affinity for the protein of interest.

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 provide reproducibility, flexibility, and convenience, thus minimizing variation during the enrichment step.

One of the major advantages of Trap products are the protocols which contain full descriptions of the components of each product. They also contain an optimization guide and recommended elution buffers to enable the direct analysis of the eluates with electrophoresis or LC-MS. SpinTrap columns require only a standard microcentrifuge. MultiTrap 96-well filter plates allow sample preparation by centrifugation or vacuum, either operated manually or automated using robotics.

Specific coupling of antibodies

Protein G Sepharose High Performance has high affinity for the Fc region of antibodies in a variety of species (Table 2). Note that protein G has affinity for a wider range of antibody species than protein A. The protocols provided with the products offer two methods for antibody attachment, allowing the enriched protein to either be eluted together with the antibody (classic protocol) or separately (cross-link protocol). Elution can be performed using the buffers described in Table 3.

Table 3. Suggested elution buffers for various situations in protein enrichment/immunoprecipitation [1]

Glycine/HCl, 1-2 M urea, pH 2.5–3.5	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 2.5-3.5	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 2.5-3.5	Many antibody-antigen bonds are broken and sufficient elution is usually achieved. This buffer has performed well when used in the classic protocol in our labs.
0.1 M ammonium hydroxide	A basic elution buffer used, for instance, when the protein of interest is acid labile.

[1] For further suggestions regarding protocol optimization, see Instructions 28-9067-72 (Protein G HP SpinTrap) and 28-9067-73 (Protein G HP MultiTrap)

Easy-to-use protocols enable reliable protein enrichment

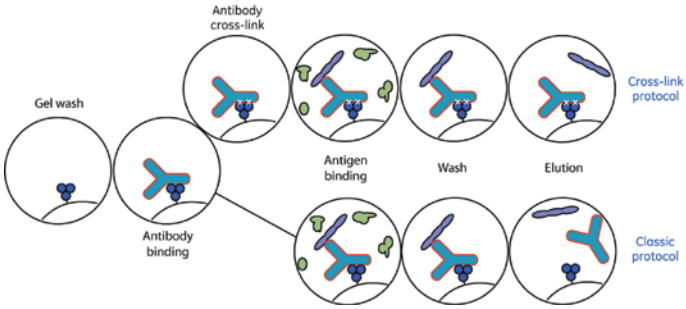


Fig 3. Schematic representation of the alternative cross-link and classic protocol approaches for use with Protein G HP SpinTrap and Protein G HP MultiTrap.

Cross-link protocol for protein enrichment

The major advantage of the cross-link protocol is that the antibody is covalently bound to the Protein G Sepharose High Performance medium, which enables the elution of the protein without co-elution of the antibody. This is often advantageous since the antibody is generally in excess compared to the protein of interest. High levels of antibody in the eluted fractions can obscure the desired signal from the protein of interest.

To demonstrate the functionality of the cross-link protocol using Protein G HP SpinTrap, human transferrin was enriched from a background of *E. coli* protein sample[1]. The transferrin concentration 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 polyclonal rabbit anti-human transferrin that was coupled to the Protein G ligand.

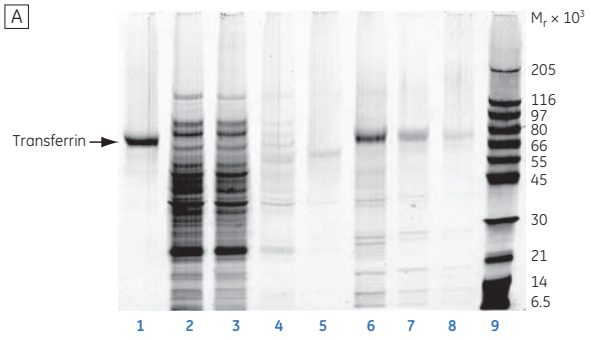
As shown in Figure 4, the majority of the enriched protein was eluted in the first elution step. In proteomics applications, it is often desirable to achieve the highest possible concentration of the protein of interest since sensitivity and detection limits are frequently limiting factors.

Reproducibility of Protein G HP SpinTrap is indicated by the analysis of the recovery (percentage of start material) of three replicates that were run in parallel (Fig 4B). Quantitation of eluted protein of interest was performed using a standard curve with known amounts of transferrin (data not shown). Essentially no unwanted protein was bound to the medium after five washing steps, as indicated by the fact that very low amounts of protein were detected in the fifth washing step (Fig 4A, lane 5). In this particular setup,

[1] In all examples described in this Data File, model proteins were enriched after being added to *E. coli* protein sample.

the enrichment of transferrin was approximately 150 to 200-fold relative to the start material using the cross-link protocol. Variation between spin columns (relative standard deviation) is generally below 15% with respect to purity and below 10% with respect to recovery (see Data File 28-9067-91 AA, Streptavidin HP SpinTrap and Streptavidin HP MultiTrap).

Trap product:	Protein G HP SpinTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 µg/ml human transferrin
Sample volume:	0.2 ml
Antibody:	Polyclonal rabbit anti-human transferrin
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



Lanes

1. Pure human transferrin (1.5 µg), Mr 77 000
2. Flowthrough (diluted 1:30)
3. First wash (diluted 1:10)
4. Third wash
5. Fifth wash
6. First elution
7. Second elution
8. Third elution
9. Protein Molecular Weight Standards (broad range)

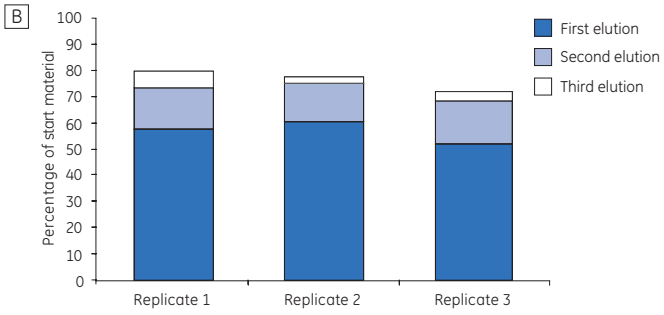
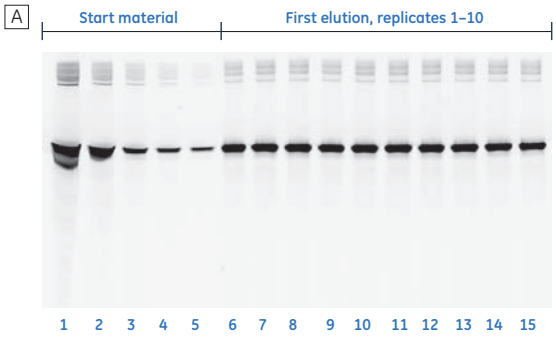


Fig 4. Enrichment of transferrin from *E. coli* cell lysate using the cross-link protocol for Protein G HP SpinTrap. Fractions were collected from every step of the enrichment process and analyzed by **(A)** SDS-PAGE (wash steps 2 and 4 have been omitted from the gel). The gel was poststained with Deep Purple Total Protein Stain and scanned using Ettan™ DIGE Imager. The result from one of three replicates is shown on the gel. **(B)** Recovery of transferrin relative to the amount of start material. The majority of protein of interest eluted in the first elution step.

The cross-link protocol also enables highly reproducible results on Protein G HP MultiTrap. Analysis of 10 parallel replicates in the enrichment of human serum albumin (HSA) from *E. coli* cell lysate shows the reproducibility of the cross-link protocol with Protein G HP MultiTrap (Fig 5). Well-to-well variation was below 10% (relative standard deviation) for both purity and recovery.

Trap product:	Protein G HP MultiTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 µg/ml human serum albumin (HSA)
Sample volume:	0.2 ml
Antibody:	Polyclonal rabbit anti-human albumin
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



Lanes

1. Start material
2. Start material (diluted 1:2)
3. Start material (diluted 1:4)
4. Start material (diluted 1:8)
5. Start material (diluted 1:16)

6–15. First elution, 10 replicates

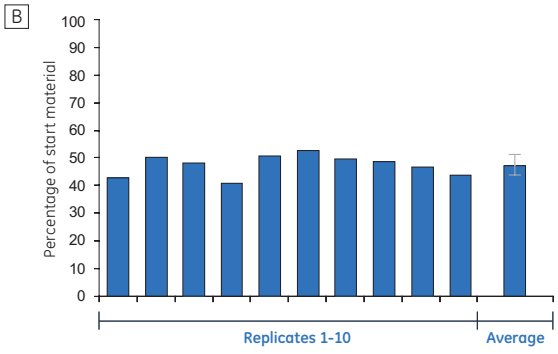


Fig 5. Enrichment of HSA from *E. coli* cell lysate using the cross-link protocol for Protein G HP MultiTrap. **(A)** Analysis by SDS-PAGE of 10 replicates of the first elution step. For visualization purposes, the HSA protein was labeled with CyDye DIGE Fluor Cy5 minimal dye. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager. **(B)** Recovery of total loaded material in the first elution step varied by 8% (relative standard deviation), illustrated by the error bar on the column showing the average of the 10 replicates.

Classic protocol for protein enrichment

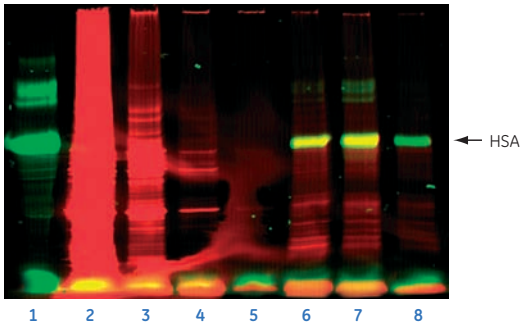
The major advantage of the classic protocol is that it is very convenient and fast since no coupling reaction needs to be performed. The price paid for the time-saving aspect of the classic protocol is co-elution of the antibody with the protein of interest. In many cases this is acceptable, for example, when sample is labeled with CyDye™ or radiolabeled. In these instances, only labeled proteins will be visualized through the respective detection procedures leaving IgG molecules undetected. Other instances when the classic protocol can be applied are when the protein of interest is of significantly different size than IgG, enabling the separate analysis of the protein of interest through, for example, electrophoresis.

To demonstrate the efficiency of the classic protocol, HSA was enriched from a background of *E. coli* protein sample (concentration of protein of interest was 0.15%) using Protein G HP SpinTrap. Analysis by SDS-PAGE of collected fractions from the spin column run revealed a significant enrichment of the protein (Fig 6).

Figure 7 shows analysis by SDS-PAGE of the enrichment of HSA using the classic protocol for Protein G HP MultiTrap. In addition to the Cy3™ and Cy5™ scanning, the gel was poststained with Deep Purple™ Total Protein Stain revealing the eluted antibodies on the gel. HSA is larger than the IgG heavy chain (reducing conditions), which enabled detection by total protein-staining techniques.

Although the classic protocol is fast and convenient, it is more sensitive to harsh washing conditions compared to the cross-link protocol. This is due to the fact that the interaction between the Fc part of the antibody and protein G is sensitive to urea and acidic conditions. In this particular experiment, only Tris buffered saline (TBS), pH 7.5 was used as wash buffer.

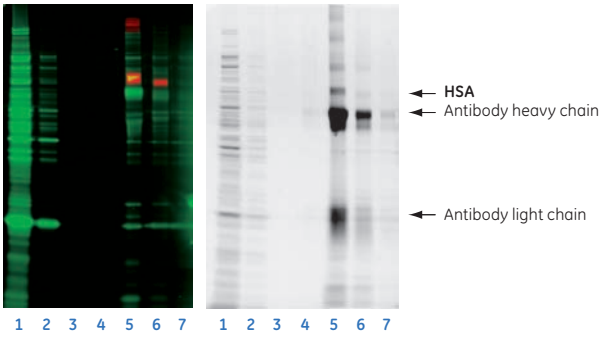
Trap product:	Protein G HP SpinTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 µg/ml human serum albumin (HSA)
Sample volume:	0.2 ml
Antibody:	Polyclonal rabbit anti-human albumin
Binding and wash buffer:	Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Elution buffer:	0.5 M acetic acid



Lanes
1. Pure HSA (1.5 µg, Cy5 labeled), M_r 65 000
2. Flowthrough (diluted 1:30)
3. First wash (diluted 1:10)
4. Third wash
5. Fifth wash
6. First elution
7. Second elution
8. Third elution

Fig 6. Enrichment of CyDye labeled (CyDye DIGE Fluor minimal dye) HSA from *E. coli* cell lysate using the classic protocol for Protein G HP SpinTrap. Fractions were collected from every step of the enrichment process and analyzed by SDS-PAGE (wash steps 2 and 4 have been omitted from the gel). For visualization purposes, *E. coli* protein was labeled with Cy3 minimal dye while HSA was labeled with both Cy3 and Cy5 minimal dyes. The HSA reference (lane 1) was only labeled with Cy5 minimal dye. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager. The antibody was not labeled and is therefore not visible in the image.

Trap product:	Protein G HP MultiTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 µg/ml human serum albumin (HSA)
Sample volume:	0.2 ml
Antibody:	Polyclonal rabbit anti-human albumin
Binding and wash buffer:	Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Elution buffer:	0.5 M acetic acid



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

Fig 7. Enrichment of HSA from *E. coli* cell lysate using the classic protocol for Protein G HP MultiTrap. Fractions were collected from every step of the enrichment process and analyzed by SDS-PAGE (wash steps 2 and 4 have been omitted from the gel). For visualization purposes, the *E. coli* protein was labeled with CyDye DIGE Fluor Cy3 minimal dye while HSA was labeled with both Cy3 and Cy5 minimal dyes. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager (left image). The antibody was not labeled and is therefore not visible in the left image. The gel was also poststained with Deep Purple Total Protein Stain and scanned using Ettan DIGE Imager to visualize total protein including antibodies (right image).

An additional advantage of the classic protocol compared to the cross-link protocol is that a larger part of the protein of interest is often found in the early elution fractions. This is exemplified in Figure 8 where approximately 80% of the total recovery is found in the first elution step. In concurrence with the cross-link protocol (Fig 5), a high level of reproducibility is obtained with Protein G HP MultiTrap; a relative standard deviation of less than 5% with respect to recovery in the first elution step was observed when using the classic protocol.

Trap product:	Protein G HP MultiTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 µg/ml human transferrin
Sample volume:	0.2 ml
Antibody:	Polyclonal rabbit anti-human transferrin
Binding and wash buffer:	Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Elution buffer:	0.5 M acetic acid

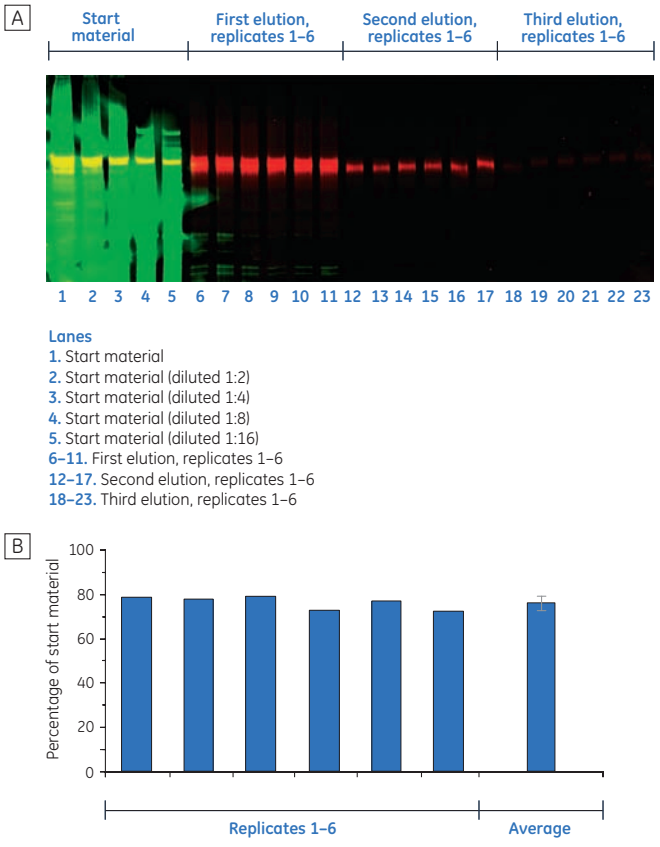


Fig 8. Enrichment of transferrin from *E. coli* cell lysate using the classic protocol for Protein G HP MultiTrap. Six replicates of the enrichment are shown. Fractions were collected from the three elution steps and analyzed by **(A)** SDS-PAGE. For visualization purposes, the *E. coli* protein was labeled with CyDye DIGE Fluor Cy5 minimal dye while the transferrin was labeled with Cy3 minimal dye. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager. The antibody was not labeled and is therefore not observed in the image. **(B)** Recovery of total loaded material in the first elution step varied by 4% (relative standard deviation), illustrated by the error bar on the column showing the average of the 10 replicates.

Ordering information

Products	Quantity	Code no.
Protein G HP SpinTrap	16 columns	28-9031-34
Protein G HP MultiTrap	4 × 96-well plates	28-9031-35
Protein A/G HP SpinTrap Buffer Kit	1	28-9135-67
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
NHS HP SpinTrap	5 ml medium and 24 empty spin columns	28-9031-28
Streptavidin HP SpinTrap	16 columns	28-9031-30
Streptavidin HP MultiTrap	4 × 96-well plates	28-9031-31
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

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imagination at work