GE Healthcare

Protein A HP SpinTrap
Protein A HP MultiTrap

Protein A HP SpinTrap™ and Protein A HP MultiTrap™ toolkits (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. Antibodies coupled to the Protein A ligand, which is immobilized on the prepacked medium, are used for the capture and enrichment of the protein of interest. The spin columns and 96-well filter plates are members of the Trap platform, which addresses the need for flexible, small-scale preparation of protein samples before downstream protein analyses such as gel electrophoresis, liquid chromatography, and LC-MS. IgG is immobilized tightly to the medium and the protein of interest can be enriched separately up to several hundred-fold dependent on the specificity of the antibody. Runs are performed in parallel, which ensures fast and reliable capture of proteins of interest from a large number of complex samples.

The key benefits of Protein A HP SpinTrap and Protein A HP MultiTrap toolkits 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.
- Choice of protocols; a classic protocol for speed or a cross-link protocol ensuring separate elution of the antibody from the protein.
- Purity; protocols support performance optimization, modification, and troubleshooting.

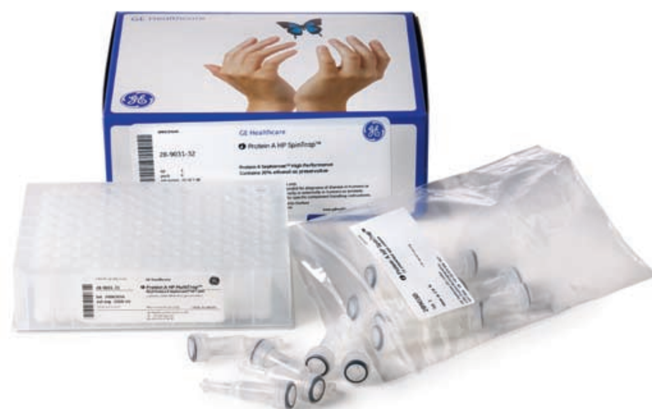


Fig 1. Protein A HP SpinTrap columns and MultiTrap 96-well filter plates are designed for efficient, small-scale enrichment of proteins of interest from clarified cell lysates and biological fluids.

Reproducible, flexible protein enrichment using Trap toolkits

Enrichment of a particular protein is often desired to increase its signal in subsequent analysis steps. Protein A HP toolkits 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 offer reproducibility, flexibility, and convenience, thus minimizing variation during the enrichment step.

Major advantages of Trap toolkits are the flexibility and reproducibility of the protocols, and full descriptions of the components of each product. In addition, Protein A HP SpinTrap and Protein A HP MultiTrap are easy to use.

Trap

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.

Characteristics of the toolkits

Protein A HP SpinTrap and Protein A HP MultiTrap are both prepacked with Protein A Sepharose™ High Performance, a proven medium with strong affinity for IgG subclasses. Reliable sample preparation is achieved on account of the 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 2). 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 toolkits.

Table 1. Characteristics of prepacked Protein A HP SpinTrap columns and Protein A HP MultiTrap 96-well filter plates

Prepacked medium	Protein A Sepharose High Performance
Matrix	Highly cross-linked agarose, 6%
Ligand	Native protein A
Ligand coupling method	N-hydroxysuccinimide activation
Ligand density	Approx. 3 mg protein A/ml medium
Binding capacity[1]	Approx. 20 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 A HP SpinTrap	
Volume of prepacked medium	100 µl
Column volume	800 µl
Column material	Polypropylene and polyethylene
Protein A 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

Specific coupling of antibodies

Protein A Sepharose High Performance has high affinity for the Fc region of antibodies in a variety of species (Table 2). The protocols provided with the toolkits offer two methods for antibody attachment, allowing the 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 2. Relative binding strengths of antibodies from various species to protein A as measured in a competitive ELISA test. Antibody binding strength to protein G is also shown for comparison. GE Healthcare also offers prepacked Protein G HP SpinTrap and Protein G HP MultiTrap; see Ordering information for details.

Species	Subclass	Protein A binding	Protein G 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

Table 3. Suggested elution buffers for various situations[1]

Glycine/HCl, 1-2 M urea, pH 2.5–3.5	Most antibody-antigen bonds are broken and sufficient elution is often 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 often 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 often 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 28-9067-70 (Protein A HP SpinTrap) and 28-9067-71 (Protein A HP MultiTrap)

Easy-to-use protocols enable reproducible protein enrichment

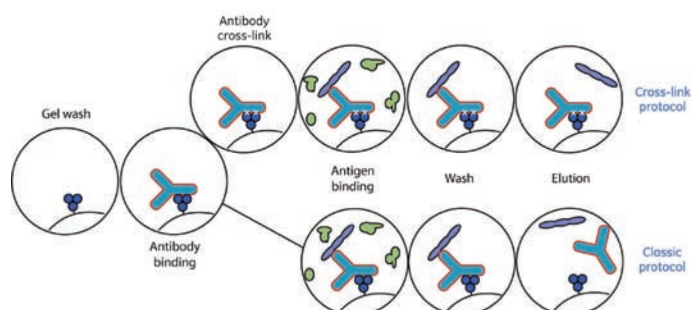


Fig 2. Schematic representation of the alternative cross-link and classic protocol approaches for use with Protein A HP SpinTrap and Protein A HP MultiTrap.

Cross-link protocol

The major advantage of the cross-link protocol is that the antibody is covalently bound to the Protein A 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 A 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

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

Trap toolkit:	Protein A 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

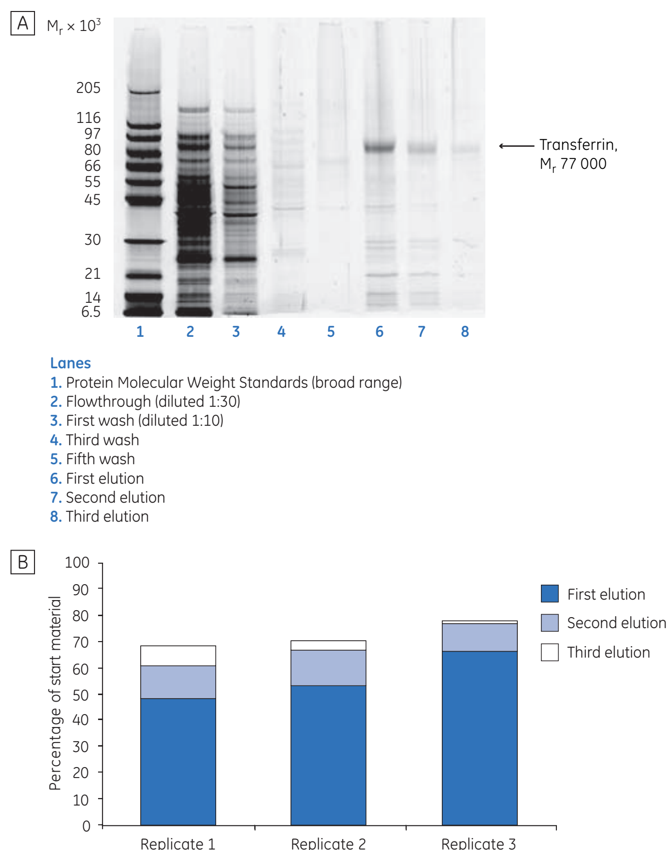


Fig 3. Enrichment of transferrin from *E. coli* cell lysate using the cross-link protocol for Protein A HP SpinTrap. Fractions were collected from every step of the enrichment process and analyzed by **(A)** SDS-PAGE (wash steps 2 and 4 have been omitted from the gel). The result from one of the three replicates is shown. The gel was poststained with Deep Purple Total Protein Stain and scanned using Ettan™ DIGE Imager. **(B)** Each elution step was quantitated using a standard curve of known amounts of transferrin. Quantitation of each elution step was performed in triplicate.

medium-abundant protein. Capture of the protein of interest was achieved using polyclonal rabbit anti-human transferrin that was immobilized to the protein A ligand.

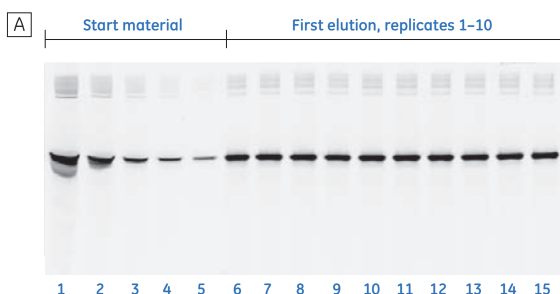
As shown in Figure 3, the majority of the enriched protein was eluted in the first elution step. In proteomics applications, it is often desirable to achieve the highest possible concentration of the protein of interest since sensitivity and detection limits are frequently limiting factors.

Reproducibility of Protein A HP SpinTrap is indicated by the analysis of the recovery (percentage of start material) of three replicates that were run in parallel (Fig 3B). 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 3A, lane 5). In this particular setup, an enrichment in the range of 200-fold was achieved 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).

The cross-link protocol also enables highly reproducible results on Protein A HP MultiTrap. Analysis of 10 parallel replicates in the enrichment of human serum albumin (HSA) from *E. coli* lysate shows the reproducibility of the cross-link protocol used with Protein A HP MultiTrap (Fig 4). Well-to-well variation was below 10% (relative standard deviation) for both purity and recovery.

Trap toolkit:	Protein A HP MultiTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 µg/ml 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 step, 10 replicates

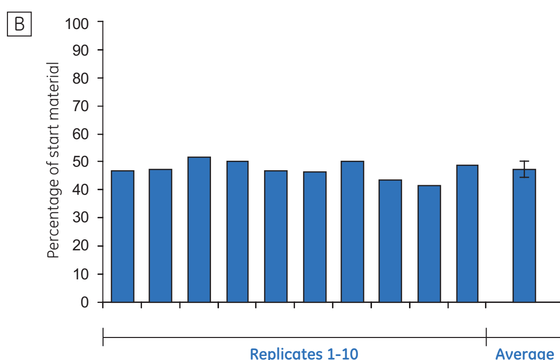


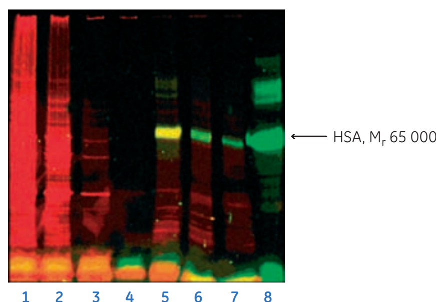
Fig 4. Enrichment of HSA from *E. coli* cell lysate using the cross-link protocol for Protein A HP MultiTrap. **(A)** Analysis by SDS-PAGE of 10 replicates of the first elution step. For visualization purposes, the HSA antigen was labeled with CyDye DIGE Fluor Cy5 minimal dye. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager. **(B)** Percentage recovery relative to the start material in the first elution step. The average value is indicated to the right in the graph and the relative standard deviation of the 10 replicates is indicated by the error bar.

Classic protocol

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 A HP SpinTrap. Analysis by SDS-PAGE of collected fractions from the spin column run revealed a significant enrichment of the protein (Fig 5).

Trap toolkit:	Protein A HP SpinTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 µg/ml 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

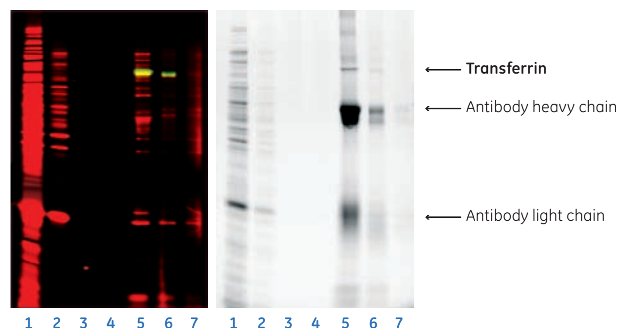


Lanes
1. Flowthrough (diluted 1:30)
2. First wash (diluted 1:10)
3. Third wash
4. Fifth wash
5. First elution
6. Second elution
7. Third elution
8. Pure HSA (1.5 µg, Cy3 labeled)

Fig 5. Enrichment of CyDye labeled (CyDye DIGE Fluor minimal dye) HSA from *E. coli* lysate using the classic protocol for Protein A 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 and HSA was labeled with both Cy3 and Cy5 minimal dyes. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager. The antibody was not labeled and is therefore not observed in the image.

Figure 6 shows analysis by SDS-PAGE of enrichment of transferrin using the classic protocol for Protein A 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. Transferrin is larger than IgG heavy chain (reducing conditions), which enabled detection by total protein-staining techniques.

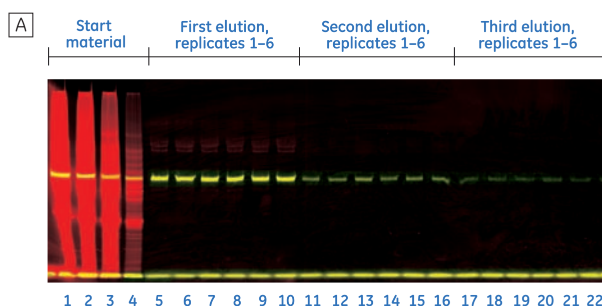
Trap toolkit:	Protein A HP MultiTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 μg/ml human transferrin
Sample volume:	0.2 ml
Antibody:	Polyclonal rabbit anti-human transferrin
Binding and wash buffer:	Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Elution buffer:	0.5 M acetic acid



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 6. Enrichment of transferrin from *E. coli* cell lysate using the classic protocol for Protein A 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 Cy5 minimal dye while the transferrin 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 observed in this image. The gel was also poststained with Deep Purple Total Protein Stain to visualize total protein including antibodies (right image).

Trap toolkit:	Protein A HP MultiTrap
Sample:	5 mg/ml *E. coli* protein containing 7.5 μg/ml 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. Start material
2. Start material (diluted 1:2)
3. Start material (diluted 1:4)
4. Start material (diluted 1:8)

5–10. First elution, replicates 1–6
11–16. Second elution, replicates 1–6
16–22. Third elution, replicates 1–6

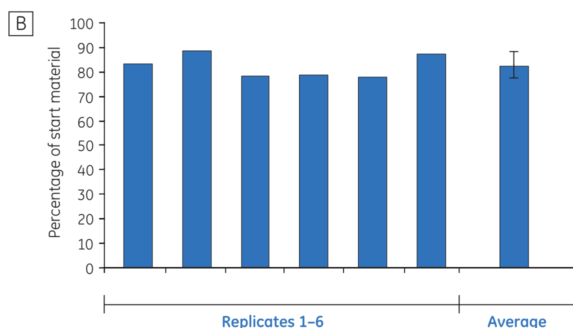


Fig 7. Enrichment of HSA from *E. coli* cell lysate using the classic protocol for Protein A HP MultiTrap. Six replicates of the enrichment are shown. Fractions were collected from the three elution steps and analyzed by **(A)** SDS-PAGE. For visualization purposes, the *E. coli* protein was labeled with CyDye DIGE Fluor Cy5 minimal dye and HSA was labeled with both Cy3 and Cy5 minimal dyes. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager. The antibody was not labeled and is therefore not observed. **(B)** Average recovery of HSA after enrichment.

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 A is sensitive to urea and acidic conditions. In this particular experiment, only Tris buffered saline (TBS), pH 7.5 was used as wash buffer.

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 7 where approximately 80% of the total recovery is found in the first elution step. In concurrence with the cross-link protocol (Fig 4), a high level of reproducibility is obtained with Protein A HP MultiTrap; a relative standard deviation of less than 5% with respect to recovery in the first elution step was observed.

Label-independent enrichment in protein analysis workflows

In protein analysis workflows, CyDye labeling techniques are often used to enable protein quantitation and minimize experimental variation. An investigation was therefore performed to determine the usability of Protein A HP SpinTrap when comparing Cy3 and Cy5 differentially labeled protein samples.

In the study, human transferrin, labeled with CyDye DIGE Fluor Cy3 and Cy5 minimal dyes, was enriched from *E. coli* lysate. Labeled transferrin was added to the lysate at various ratios ranging from 1:3 to 2:1 with respect to Cy3 and Cy5 labels. The protein of interest was enriched according to the classic protocol and Cy3:Cy5 ratio differences were analyzed and quantitated (Fig 8). Expected levels of Cy3 and Cy5 labeled protein corresponded well to the measured levels of labeled protein (R^2 > 0.99) demonstrating that Protein A SpinTrap performed equally well for both the Cy3 and Cy5 labeled protein of interest (Fig 8B).

Trap toolkit:	Protein A HP SpinTrap
Sample:	5 mg/ml *E. coli* protein containing various ratios of Cy3:Cy5 labeled 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, pH 7.5
Elution buffer:	0.1 M glycine/HCl, pH 2.8

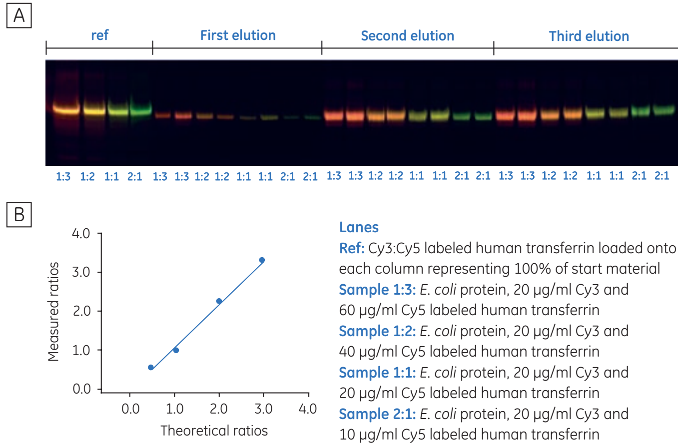


Fig 8. Enrichment of transferrin labeled with CyDye DIGE Fluor Cy3 or Cy5 minimal dye from *E.coli* cell lysate at different ratios ranging from 1:3 to 2:1 with respect to Cy3 and Cy5 labeled protein. Protein A HP SpinTrap was used applying the classic protocol for the enrichment. **(A)** Samples were collected from the three elution steps and analyzed by SDS-PAGE. After electrophoresis, the gel was scanned directly using Ettan DIGE Imager (antibody and *E. coli* protein were not labeled and are therefore not observed). **(B)** Quantitation of Cy3 and Cy5 labeled transferrin enriched on the SpinTrap columns was performed using ImageQuant™ TL software. Measured values in the first elution steps were compared to theoretical values.

Ordering information

Products	Quantity	Code no.
Protein A HP SpinTrap	16 columns	28-9031-32
Protein A HP MultiTrap	4 × 96-well plates	28-9031-33
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 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
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

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