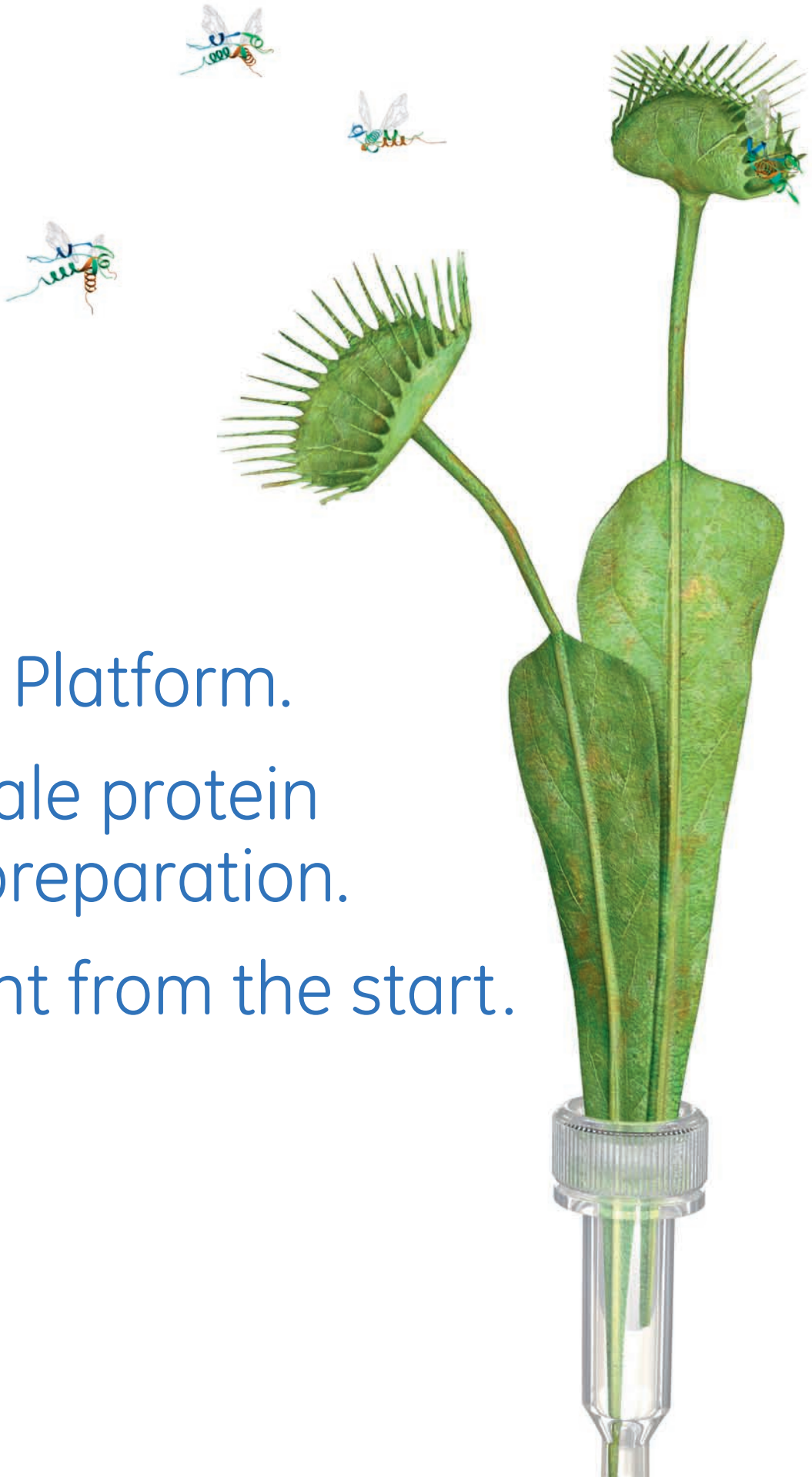


The Trap Platform.

Small-scale protein sample preparation.

Get it right from the start.

Product Profile

The small-scale preparation of protein samples

Optimized for downstream applications

The small-scale preparation of protein samples is key to the success and quality of subsequent downstream experimental work and analyses. This critical aspect of protein science has hardly changed for decades and is increasingly regarded as a major limiting step in the study of proteins.

The continuous development of the Trap platform is designed to bring total solutions to the ever increasing demands of the protein researcher.

Building on the experience and knowledge of proteins gained over decades in our laboratories together with the heritage from the HiTrap™ platform of protein capture products, the Trap platform is being developed further with the introduction of new SpinTrap™, GraviTrap™, and MultiTrap™ formats for various steps in protein sample preparation.

The Trap platform is a range of application-based protein preparation products designed to enable researchers to achieve **high reproducibility, yield, and purity** of their proteins in line with the specific demands of downstream techniques or analytical methods, e.g. 1-D, 2-D electrophoresis, Western blotting, liquid chromatography, protein interaction, and mass spectrometry.

Optimization guides help you achieve maximum performance for your specific application and for the characteristics of your protein of interest. All Trap protocols are completely transparent. Full information about formats, media, buffers, and methodologies is provided to support and enable further optimization by the researcher.

The current Trap product range includes products for tagged protein preparation, desalting, and protein enrichment. Continuous development of the platform will extend this range to cover all protein preparation needs.

Find the right protein sample preparation product for your application in this Product Profile or in the Protein Sample Preparation Selection Guide at:

www.gehealthcare.com/trap

Trap

HiTrap

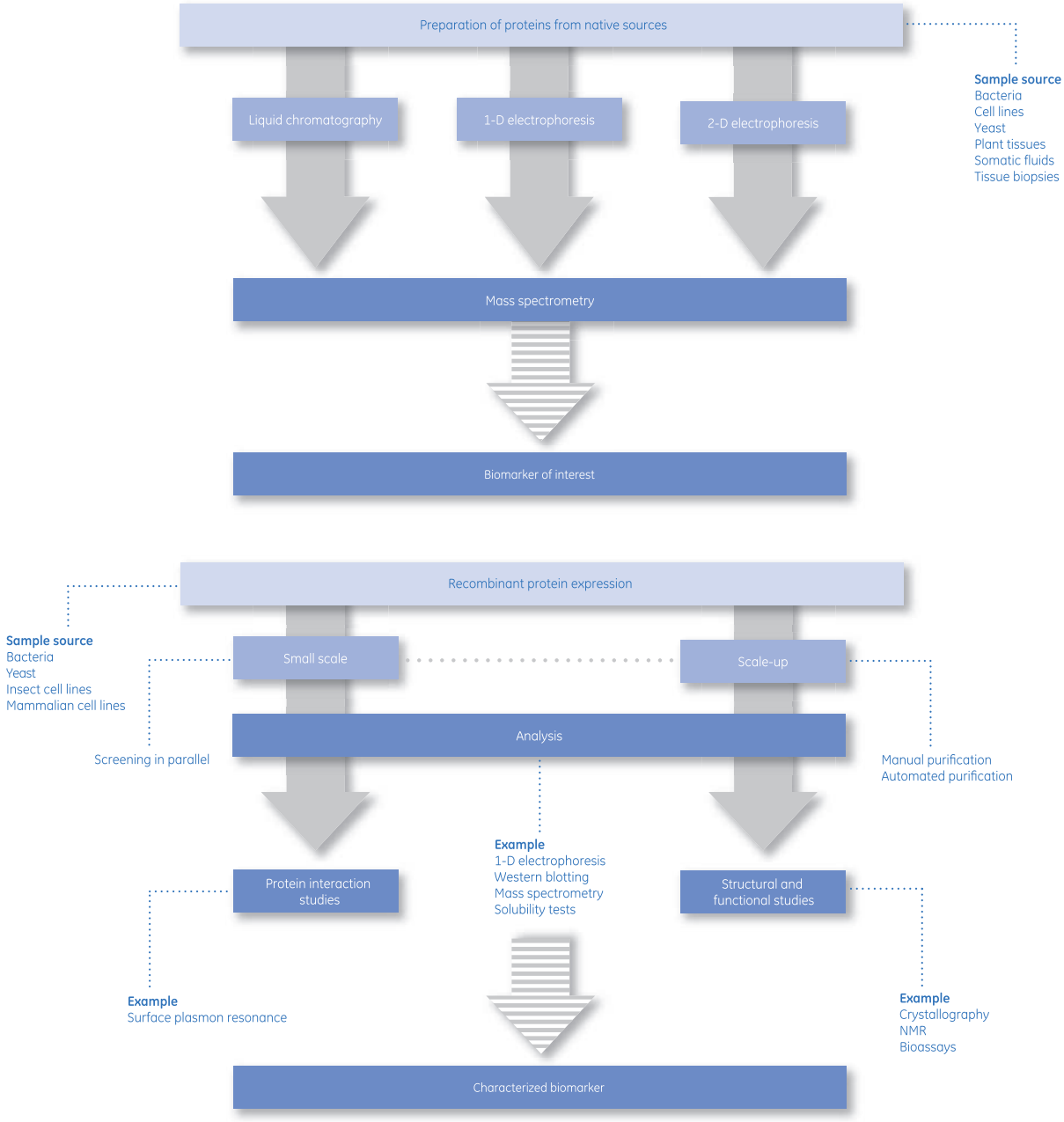
GraviTrap

SpinTrap

MultiTrap

Application-focused product selection

The Trap platform focuses on optimizing protein sample preparation for typical downstream applications. The selection guide at www.gehealthcare.com/trap makes choosing the right product easy – simply click on the arrow pointing towards your intended analysis method.



Parallel affinity-based sample preparation

Enabling top-down LC-MS protein analysis

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GE Healthcare, Uppsala, Sweden

Protein G HP MultiTrap was used to enrich human α-transferrin (hTf) more than 100-fold from *E. coli* extract. The eluate resulting from enrichment was analyzed by SDS-PAGE or LC-MS, and the yield was sufficient for extensive MS analysis.

Introduction

The complexity and abundance of proteins in most biological systems exceed the resolution capacity of every currently existing analytical technique. Perhaps the most striking example is the serum proteome, which features extreme differences in protein concentrations, with the proteins considered the most interesting often ten orders of magnitude less abundant than albumin or immunoglobulins.

Protein analyses are commonly used to study various disease or treatment states. To obtain statistically relevant data a number of biological and experimental replicates are needed, leading to a large number of samples. Preparing the samples for these studies is tedious work and a source of error. The demand for highly reproducible sample preparation approaches is increasing as protein analysis and proteomics begin to address actual biological questions, and the need for convenient and reproducible sample preparation methodologies to reduce sample complexity is larger than ever.

Protein fractionation

A proteome can be fractionated in different ways depending on the protein properties that are of interest. The purpose of the sample preparation procedure is to make the biological sample, for example, blood, plasma, tissue, urine, cell culture, plant extract, or bacteria, manageable enough to enable an informative characterization of the protein(s) of interest.

Affinity- and immunocapture-based methods are well-established to enrich subsets or individual proteins of interest. With these approaches, sample complexity can be reduced so that a simple one-dimensional separation procedure such as electrophoresis or liquid chromatography (LC), may be sufficient to resolve captured protein constituents. The typical end-point of a proteomic analysis is identification by mass spectrometry (MS).

Parallel immunocapture preparation and analysis

Protein G HP MultiTrap is one of a new range of products for protein enrichment by immunocapture prior to downstream protein analyses such as SDS-PAGE and LC-MS. To demonstrate the functionality of Protein G HP MultiTrap, human transferrin (hTf) was enriched from *E. coli* cell containing hTf added to a concentration of 0.15% of the total protein content, which approximately corresponds to the concentration of a medium-abundant protein. To enable rapid downstream visualization and protein quantitation, hTf was labeled with CyDye™ DIGE Fluor Cy5™ minimal dye prior to mixing. Rabbit polyclonal anti-hTf was immobilized in the wells of a Protein G HP MultiTrap filter plate, and the antibody-protein G complexes were cross-linked using dimethyl pimelimidate dihydrochloride. The *E. coli* sample was added and allowed to incubate before wash and elution. SDS-PAGE analysis of three elution fractions from six replicates originating from two different multiwell plates is shown in Figure 2.

The majority of the enriched hTf was eluted in the first fraction with a recovery above 50% and an enrichment factor of more than 100 relative to the starting material. This procedure proved to be highly reproducible with relative standard deviations well below 10% for both target protein recovery and specific purity.

Reversed-phase LC-MS analyses were performed and the starting material and hTf-enriched elution fraction were compared. Start material was diluted with the elution buffer to a suitable protein concentration and thereafter treated the same way as the enriched sample. Tricarboxyethylphosphine was added to reduce the disulfide bonds of the proteins and cysteines were alkylated with iodoacetamide. After alkylation, the proteins were cleaved into smaller fragments by addition of stabilized porcine trypsin and incubated overnight at room temperature. Ten microliter of each sample was injected on a C18 enrichment column and desalted on-line using the Ettan™ MDLC chromatography system. Bound peptides were then separated on an analytical C18 reversed-phase column (0.075 × 150 mm) with a gradient from 0 to 67% (v/v) acetonitrile in 0.1% formic acid and water during 60 min at a flow rate of 200 nl/min. The effluent was sprayed into the nanoflow electrospray source of an ion trap mass spectrometer. An automatic data-dependent scan method was used to acquire MS and MS/MS spectra, and an automated protein database search completed the identification of the peptide fragments as well as the overall identification of proteins. Human and *E. coli* databases were used in the search, allowing for the two modifications — oxidized methionines and carboxyamido-methylated cysteines.

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Proteins detected

About 50 different proteins were detected and identified with confidence ($p<0.01$) in the start material. These proteins were mainly high-abundant *E. coli* proteins, including proteins involved in the protein synthesis machinery, metabolic enzymes, and various heat-shock proteins (Fig 3). They were generally identified by only one or two peptide fragments. hTf was not detected in the start material. In contrast, the identification of proteins in the enriched sample showed hTf as the major protein hit with high confidence, represented by 48 unique hTf-derived peptide fragments covering 70% of the precursor sequence (Fig 3). Spectra were obtained with signal strength that permitted detailed information extraction. The remaining proteins identified were either ribosomal proteins or proteins closely associated with the ribosomal complex. Notably, the yield in this particular experiment was high enough to allow the use of only 5% of the first eluted fraction, indicating that protein(s) with significantly lower abundance than reviewed in this study may well be analyzed using the same protocol.

Conclusions

An immunoprecipitation sample preparation method based on Protein G HP MultiTrap proved to be convenient and highly reproducible, generating an enriched sample that could be analyzed with an SDS-PAGE or LC-MS approach. The results demonstrate the strength of reducing sample complexity prior to analysis. In addition, the yield of the enriched protein from the model system used in this study was sufficient to enable an extensive MS analysis.

Reference

1. Data File: Protein G SpinTrap and Protein G MultiTrap, GE Healthcare, 28-9067-90, Edition AA (2006).

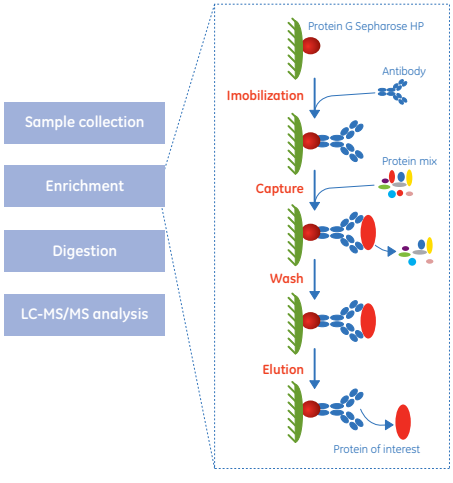


Fig 1. Applied sample preparation work flow.

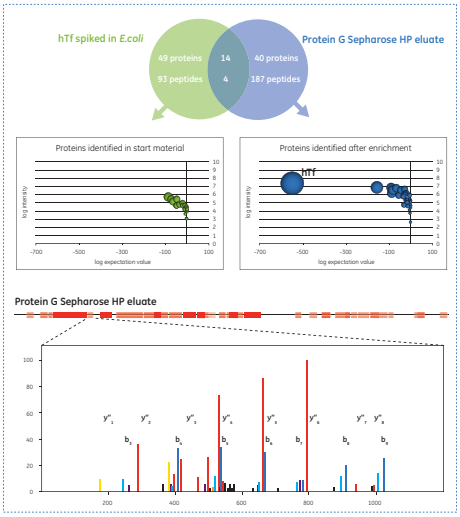


Fig 2. Enrichment of Cy5-labelled human serum transferrin (hTf) using cross-linked α-transferrin antibodies and Protein G Sepharose High Performance in a multiwell format. Start material was *E.coli* protein (5 mg/mL) containing hTf (7.5 µg/mL). Depicted is the analysis by SDS-PAGE of six replicates from three elution steps. The gel was stained by Deep Purple Total Protein Stain and scanned in the Ettan DIGE Imager using excitation and emission wavelengths specific for Cy5 (red) and Deep Purple (green), respectively.

Trap toolkit	Protein G HP MultiTrap
Sample:	5 mg/ml *E .coli* protein containing 7.5 µg/ml hTf
Sample volume:	0.2 ml
Antibody:	Polyclonal rabbit anti-hTf
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

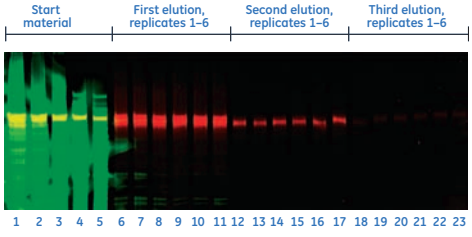


Fig 3. Proteins and peptides identified by LCMS/ MS analysis with p values less than 0.01. Upper panel: The number of unique proteins identified in each sample, i.e., start material (green) and hTf enriched material (blue), respectively. The respective protein positions in the graph relate to expectation value (X-axis) and intensity (Y-axis). The sizes of the bubbles represent the number of different peptides identified per protein. Lower panel: Detailed information on the sequence coverage of hTf (red boxes) compared to the theoretical sequence (black line), along with an example of an MS/MS spectrum of the indicated peptide with b- and y-ion series annotated.

Reproducible protein enrichment

SpinTrap enriches proteins with very low variation between replicates. Typical recovery rates vary by less than 10% (Fig 1 and 2). Similarly, MultiTrap plates allow preparation of up to 96 samples in parallel with well-to-well variation below 10% (Fig 3). SpinTrap and MultiTrap formats are designed so that methods developed in one format can be simply transferred to the other. For projects requiring high throughput, MultiTrap gives significant cost savings.

SpinTrap reproducibility

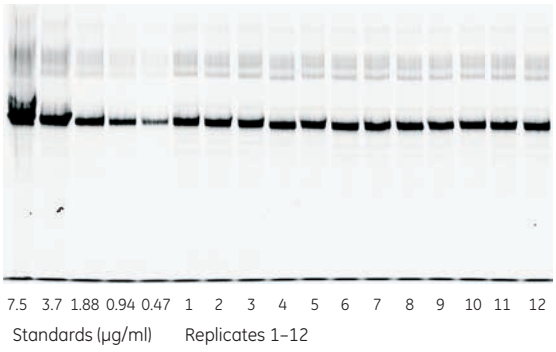


Fig 1. Enrichment of human albumin (HSA) from *E. coli* lysates (5 mg/ml *E.coli* protein + 7.5 µg/ml HSA) using Streptavidin HP SpinTrap. Twelve columns were run in parallel. Recovery from the first elution is shown. Known amounts of HSA were run as standards.

SpinTrap reproducibility

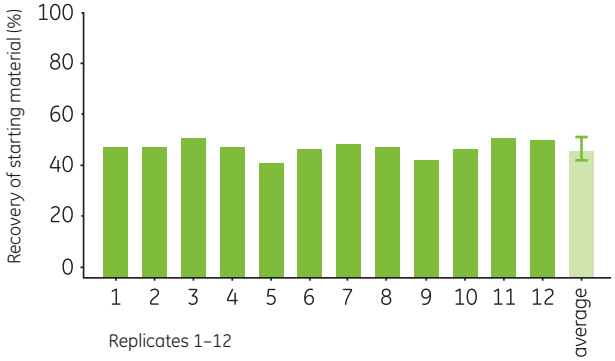


Fig 2. Recovery of total loaded material varied by 6% (relative standard deviation), illustrated by the error bar on the column showing the average of the 12 samples (red bar).

MultiTrap reproducibility

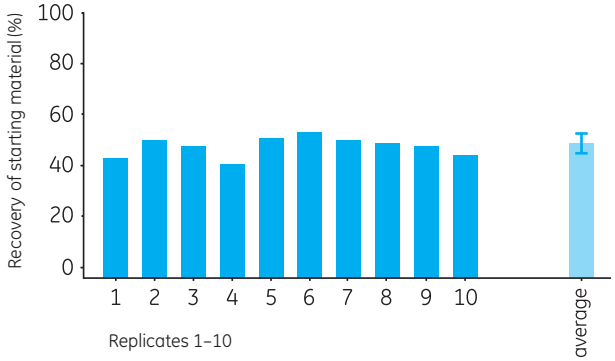


Fig 3. Enrichment of a known amount of HSA from an *E. coli* cell lysate (5 mg/ml *E. coli* protein + 7.5 µg/ml HSA) using Protein G HP MultiTrap shows high well-to-well reproducibility (relative standard deviation <10%). The protein recovery of the first elution step from 10 wells is shown.

Application-focused protocols

We've added flexibility in the form of Optimization Guides to improve performance for both specific applications and the characteristics of your protein. Beyond simply cleaning up samples, you can, for example, prepare labeled and pooled protein samples upstream of DIGE analysis. You get consistent, label-independent protein enrichment – a key requirement for quantitative expression analysis.

Preparing labeled protein samples before DIGE analysis

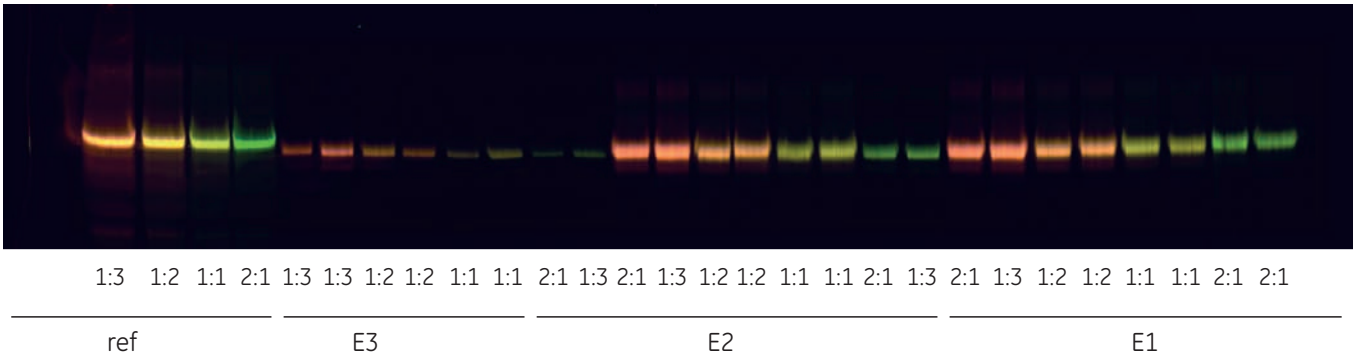
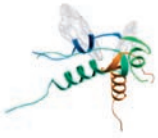


Fig 4. SpinTrap and MultiTrap products ensure consistent preparation of differentially labeled proteins. Transferrin labeled with either Cy3 or Cy5 was added in known Cy3:Cy5 ratios ranging from 1:3 to 2:1 to an *E. coli* lysate. Transferrin was then enriched using Protein A HP SpinTrap and analyzed for Cy3/Cy5 ratio differences. Samples were collected in three elution steps (E1-E3), and separated by SDS-PAGE. Ref: 100% starting material (transferrin mixed and loaded directly into the SpinTrap).



Expected vs. measured values for differential analysis (E1)

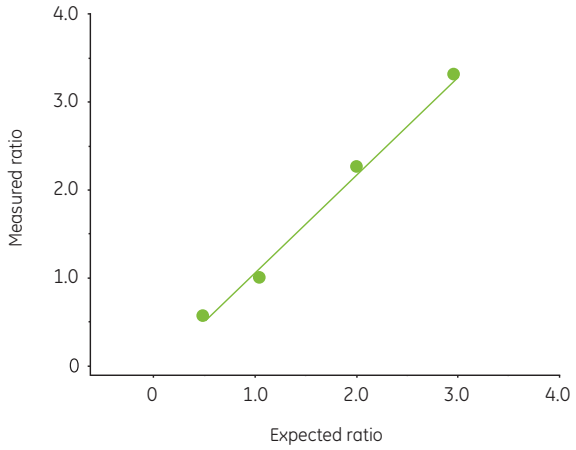


Fig 5. The measured values in the first and the second elution steps in Figure 4 correspond well to expected values ($R^2 > 0.99$).

Ordering information

Product	Format	Code no.
Tissue homogenization		
Sample Grinding Kit	Microspin columns	80-6483-37
Enzyme regulation		
Protease Inhibitor Mix	Reagent	80-6501-23
Nuclease Mix	Reagent	80-6501-42
Protein fractionation		
2-D Fractionation Kit	Reagents	80-6501-04
Protein depletion		
Albumin and IgG Removal Kit	Microspin columns	RPN6300
Protein enrichment		
NHS HP SpinTrap	SpinTrap	28-9031-28
Streptavidin HP SpinTrap	SpinTrap	28-9031-30
Streptavidin HP MultiTrap	MultiTrap	28-9031-31
Protein A HP SpinTrap	SpinTrap	28-9031-32
Protein A HP MultiTrap	MultiTrap	28-9031-33
Protein G HP SpinTrap	SpinTrap	28-9031-34
Protein G HP MultiTrap	MultiTrap	28-9031-35
Desalting/buffer exchange/clean-up		
Disposable PD-10 Desalting Columns	Gravity column	17-0851-01
Mini Dialysis Kit, 1 kDa, 250 µl	Dialysis tubes	80-6483-75
Mini Dialysis Kit, 1 kDa, 2 ml	Dialysis tubes	80-6483-94
Mini Dialysis Kit, 8 kDa, 250 µl	Dialysis tubes	80-6484-13
Mini Dialysis Kit, 8 kDa, 2 ml	Dialysis tubes	80-6484-32
2-D Clean-Up Kit	Reagents	80-6484-51
SDS-PAGE Clean-Up Kit	Reagents	80-6484-70
Antibody purification		
Ab SpinTrap	SpinTrap	28-4083-47
Ab Buffer Kit	Buffer kit	28-9030-59

Product	Format	Code no.
Histidine-tagged protein purification		
His MultiTrap FF	MultiTrap	28-4009-89
His MultiTrap HP	MultiTrap	28-4009-90
His SpinTrap	SpinTrap	28-4013-53
His GraviTrap	GraviTrap	11-0033-99
His GraviTrap Kit	GraviTrap	28-4013-51
His Buffer Kit	Buffer kit	11-0034-00
HisTrap™ HP (1 ml)	HiTrap	17-5247-01
HisTrap HP (5 ml)	HiTrap	17-5248-01
HisTrap FF (1 ml)	HiTrap	17-5319-01
HisTrap FF (1 ml)	HiTrap	17-5255-01
HisTrap FF crude (1 ml)	HiTrap	11-0004-28
HisTrap FF crude (5 ml)	HiTrap	17-5286-01
Anti-His Antibody	Reagent	27-4710-01
GST-tagged protein purification		
GST MultiTrap FF	MultiTrap	28-4055-00
GST MultiTrap HP	MultiTrap	28-4055-01
GST Purification Module	GraviTrap	27-4570-02
GST SpinTrap Purification Module	SpinTrap	27-4570-03
GST Detection Module	Reagents	27-4590-01
GST 96-Well Detection Module	96-well plate	27-4592-01
pGEX-vectors	Reagent	see www.gehealthcare.com/lifesciences for details
5′ pGEX Sequencing Primer	Reagent	27-1410-01
3′ pGEX Sequencing Primer	Reagent	27-1411-01
GSTrap™ HP (1 ml)	HiTrap	17-5281-01
GSTrap HP (5 ml)	HiTrap	17-5282-01
GSTrap FF (1 ml)	HiTrap	17-5130-02
GSTrap FF (5 ml)	HiTrap	17-5131-01
GSTrap 4B (1 ml)	HiTrap	28-4017-45
GSTrap 4B (5 ml)	HiTrap	28-4017-48
Anti-GST Antibody	Reagent	27-4577-01
PreScission™ Protease	Reagent	27-0843-01
Thrombin protease	Reagent	27-0846-01
Factor Xa thrombin protease	Reagent	27-0849-01
Miscellaneous		
MultiTrap collection plate	MultiTrap	28-4039-43

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