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Pure simplicity

Why make your job more difficult than necessary? You no longer have to be a chromatography expert to get the purity and quantity you need. Our products and applications have 50 years of experience packed into them to help keep your lab work on track.

Whether you are working with small-scale manual screening and purification tasks or large numbers of samples on chromatography systems, we can provide an unparalleled wealth of knowledge. This brochure highlights proven solutions to many of today's protein purification challenges, with examples of how and where our products can help you.

Want to know more? Visit: www.gelifesciences.com/protein-purification


GE Healthcare
28-4009-90
His MultiTrap™ FF
50 µl Ni Sepharose™ 6 Fast Flow / well
lot 310613
est exp 2007-05
store at +4 to +30°C
contains 20% ethanol as preservative
Caution! Contains nickel.
May produce an allergic reaction.
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GE Healthcare Bio-Sciences AB
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HiTrap™ 5 ml
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5 ml
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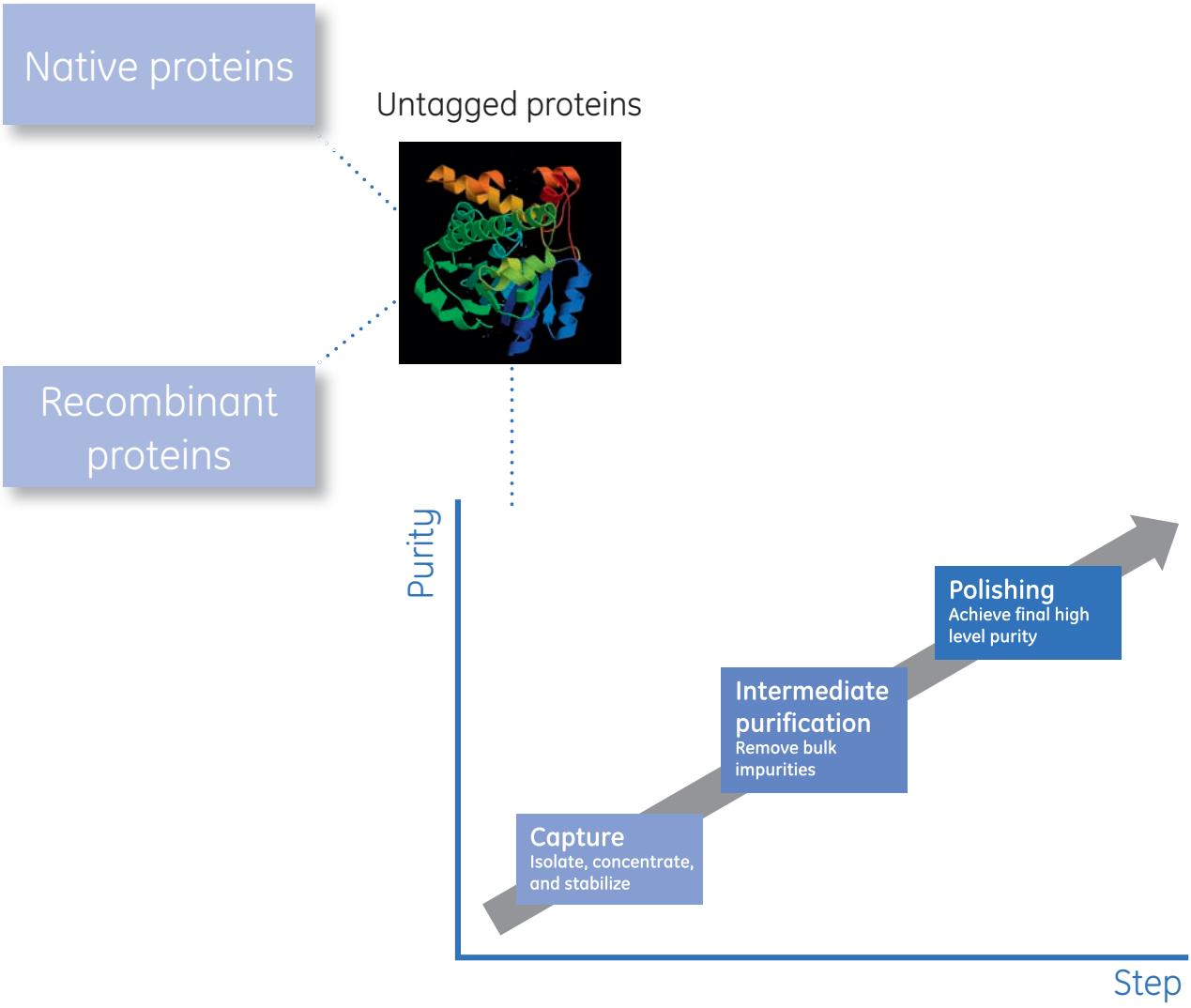
Purification of untagged proteins

Purification of native or recombinant untagged proteins can be complex if multiple purification steps are needed. The three-step purification strategy: capture, intermediate purification, and polishing (CiPP), ensures efficient purification of the target molecule. In the capture step, the objectives are to isolate, concentrate, and stabilize the target product. The product should be transferred to an environment that will conserve activity. One or several intermediate purification steps may then be required to eliminate most of the contaminants. Finally, a polishing step is needed to remove trace amounts of contaminants from the target protein. For CiPP strategy details, consult the Protein Purification Handbook 18-1132-29.

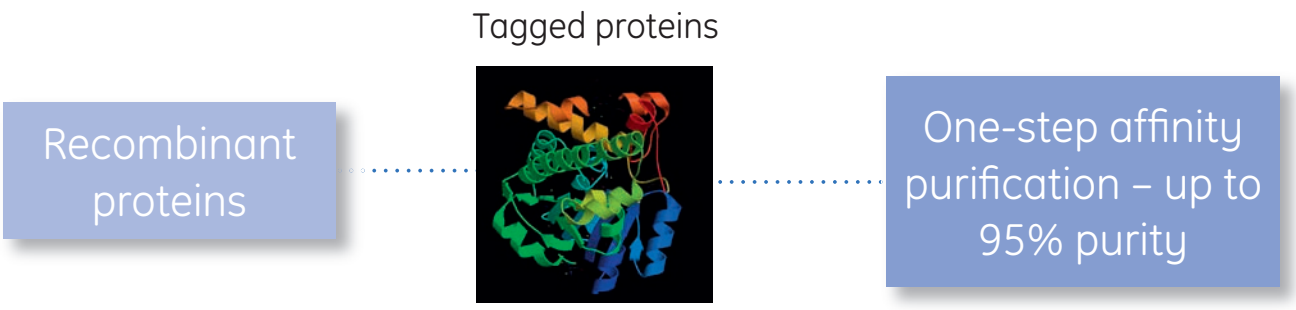
Classic purification



Purification of tagged proteins

The purification of tagged proteins is simpler and saves time due to high specificity between the tag on the expressed protein and the ligand on the affinity medium. You get high purity in a single step – affinity purification typically gives up to 95% purity. If greater purity is required further purification steps may be necessary. The Recombinant Protein Purification Handbook, 18-1142-75, covers many aspects of expression and purification of tagged recombinant proteins.

Simple purification



Histidine-tagged proteins

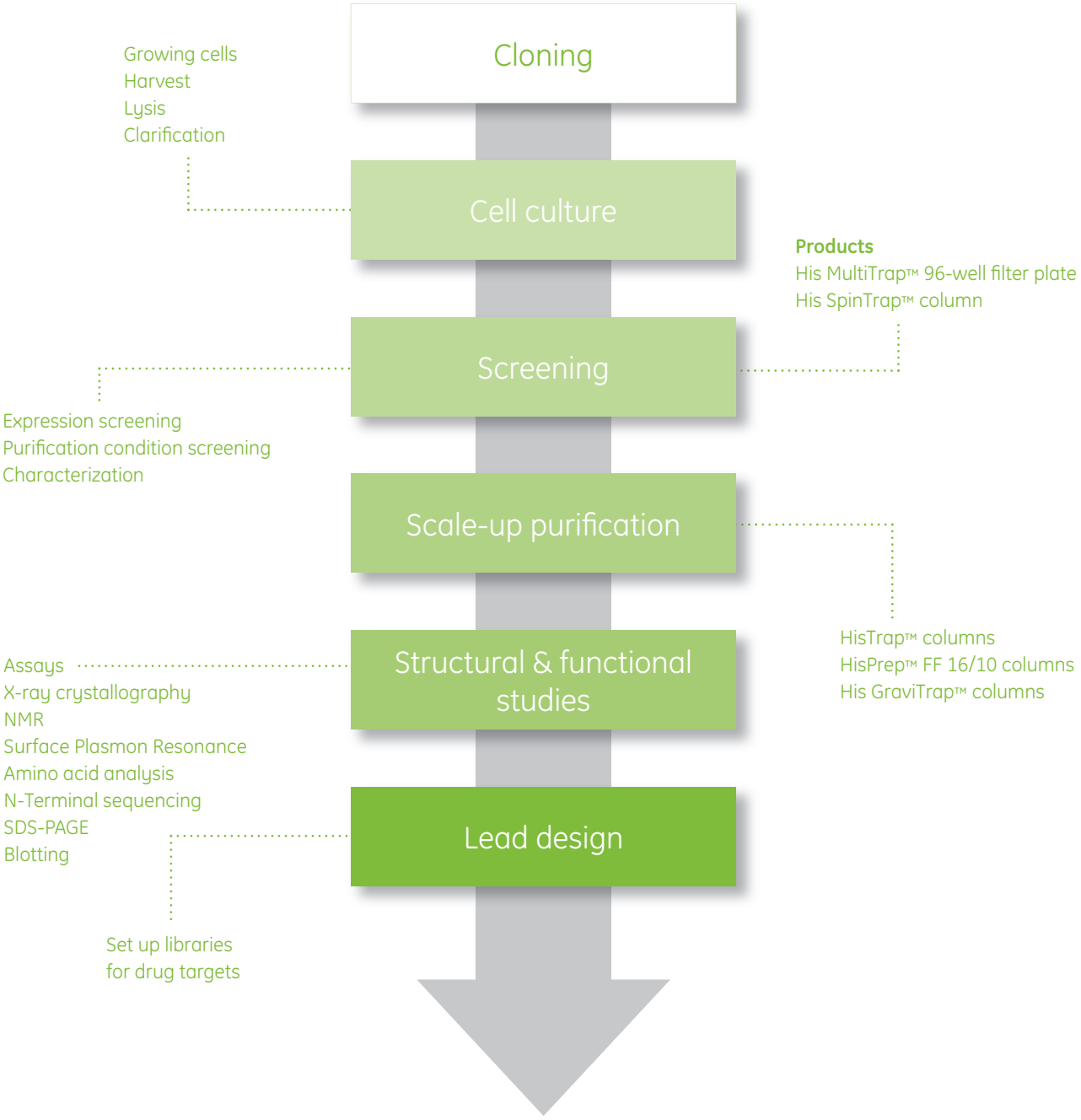
High binding capacity saves time and media/ buffer consumption

Histidine tags are widely used since they are small and rarely interfere with the function, activity, or structure of target proteins. Immobilized metal ion affinity chromatography (IMAC) is the most common method for purifying histidine-tagged proteins. IMAC media charged with divalent metal ions such as nickel selectively retains histidine-tagged proteins. It also allows the purification of insoluble histidine-tagged proteins from inclusion bodies when denaturing conditions are used.

Successful IMAC purification gives a high yield of pure and active target protein. The high binding capacity of Ni Sepharose™ media saves time and reduces costs from media and buffer consumption.

Since many proteins have intrinsic histidine and/or cysteine amino acid residues, other non-specific proteins bind to the IMAC media together with the target protein. This is noticeable when recombinant proteins have low expression levels in the host cell. In these cases, optimization of binding, wash, and elution conditions with imidazole is necessary. Increasing the concentration of imidazole in binding and wash buffer generally decreases non-specific binding, whereas lower concentrations give stronger affinity interaction. The key is finding the balance.

From gene to target protein

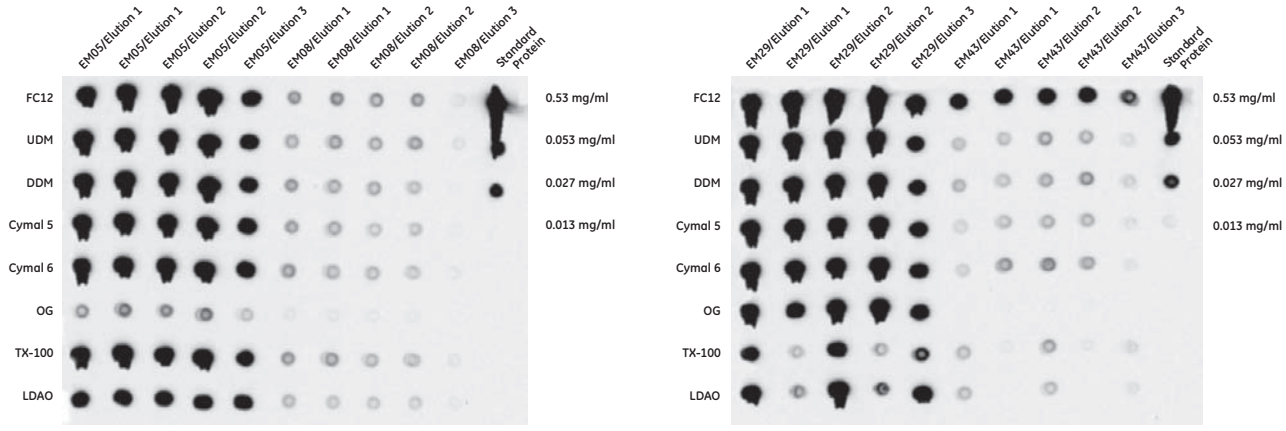


The workflow above is a typical example of a protein production process that starts at the gene and ends with a target protein. To simplify your lab work, we offer a wide range of products that fit into your workflow.

Detergent screening and purification of membrane proteins

Products featured: His MultiTrap FF, HisTrap FF crude, HiLoad 16/60 Superdex 200 pg, ÄKTAexplorer 10

Convenient and reproducible optimization of screening parameters can be performed with His MultiTrap 96-well filter plates to easily define scale-up conditions. Eight detergents were screened for their effect on the solubility of six histidine-tagged membrane proteins. Results are shown for purification screening of four proteins: putative transferase (EM05), regulatory protein (EM08), GlpG protein (EM29), and cation transporter (EM43).



Dot blots (anti-histidine immunodetection) of membrane proteins EM05, EM08, EM29, and EM43 purified on His MultiTrap FF in the presence of different detergents. Reproducibility is shown by repeats of eluates 1 and 2 dot blots, which are two independent extractions and purifications.

Detergents

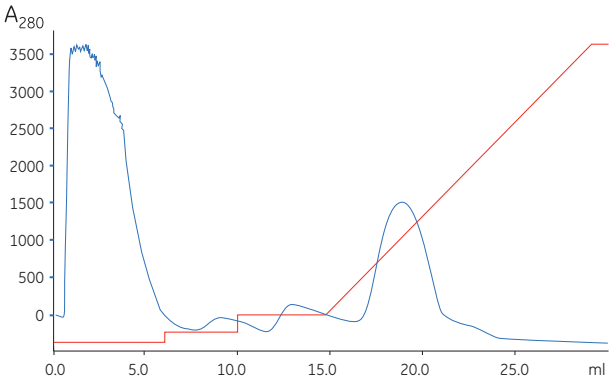
FC12 1% Fos-Choline
UMD 1% undecyl maltoside
DDM 1% dodecyl maltoside
Cymal 5 1% Cymal-5
Cymal 6 1% Cymal-6
OG 2% octyl glucoside
TX-100 1% Triton™ X-100
LDAO 1% lauryl dimethylamine oxide

Summary

Detergent screening using His MultiTrap purification followed by dot blot allowed rapid screening of four proteins and eight detergents within 60 min.

After screening with His MultiTrap, the optimal method conditions were used for scale-up in protein structure and function studies.

Capture

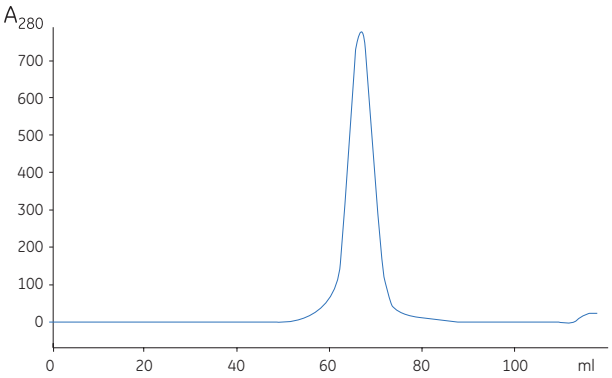


Sample: *E. coli* lysate containing histidine-tagged EM05

Columns: Capture – HisTrap FF crude 1 ml
Polishing – HiLoad™ 16/60 Superdex™ 200 pg

Affinity chromatography (AC) binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, 0.5 mM TCEP, 1% Fos-Choline 12, pH 7.4

Polishing



AC wash buffer: As binding buffer but with 40 mM imidazole

AC elution buffer: As binding buffer but with 500 mM imidazole

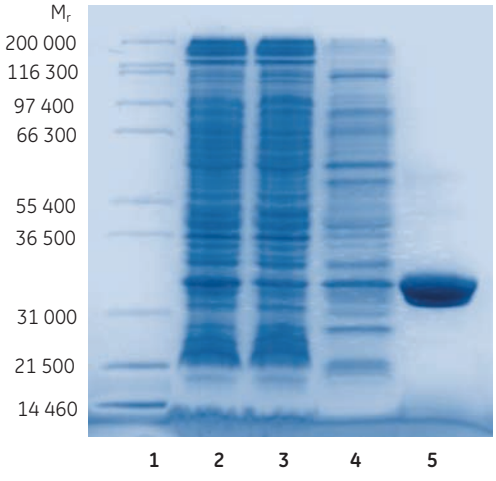
GF buffer: 20 mM Tris-HCl, 50 mM NaCl, 0.5 mM TCEP, 0.03% DDM*, pH 8.0

System: ÄKTAexplorer™ 10

* DDM was used in the polishing buffer since this detergent is the preferred choice for protein crystallization.

SDS-PAGE

Lane	
1	Molecular weight markers, M_r
2	Solubilized membranes (start material)
3	Flowthrough, HisTrap FF crude column
4	Wash, HisTrap FF crude column
5	Eluate, HiLoad 16/60 Superdex 200 pg column



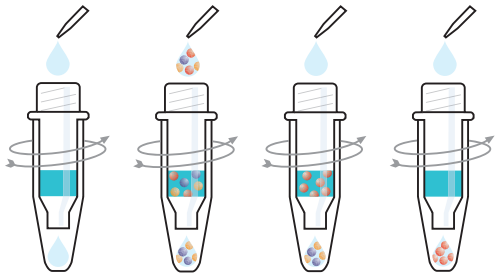
Acknowledgements: V. Lieu and S. Eshaghi, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.

Summary
The detergent selected in the screening study, 1% FC12, was successfully transferred from a His MultiTrap FF to a HisTrap FF crude column, and a further polishing step was performed by gel filtration to give a highly pure protein.

Manual small-scale optimization in 10 min

Product featured: His SpinTrap

Concentration of imidazole in the binding buffer and sample is an important factor that affects the final purity and yield of the target protein. His SpinTrap is a fast and convenient tool for determination of optimal imidazole concentrations. This was demonstrated by a series using 5, 50, 100, and 200 mM imidazole in samples and binding buffers to purify APB-7-$(His)_6$ (M_r 28 000) on His SpinTrap.



Purifying histidine-tagged proteins with His SpinTrap is a simple four-stage procedure that can be performed in 10 min using a microcentrifuge:

(1) After placing the column in a 2 ml microcentrifuge tube, equilibrate by adding binding buffer and centrifuge

(2) Add sample, centrifuge

(3) Wash with binding buffer, centrifuge

(4) Elute the target protein with elution buffer by centrifugation

SDS-PAGE

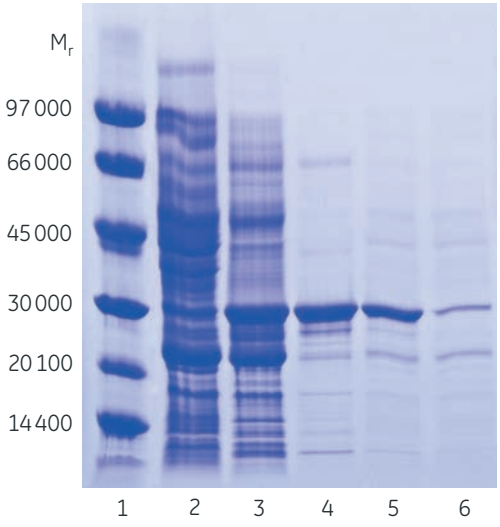
Lane	
1	Low molecular weight markers
2	Start material (diluted 1:10)
3	Eluted pool, 5 mM imidazole during binding (diluted 1:2)
4	Eluted pool, 50 mM imidazole during binding (diluted 1:2)
5	Eluted pool, 100 mM imidazole during binding (diluted 1:2)
6	Eluted pool, 200 mM imidazole during binding (diluted 1:2)

Column: His SpinTrap

Sample: 600 µl *E. coli* BL-21 lysate containing 400 µg APB 7-$(His)_6$ (pI 6, M_r 28 000, C-terminal histidine-tagged)

Binding/wash buffer: 20 mM phosphate, 500 mM NaCl, 5-200 mM imidazole, pH 7.4

Elution buffer: 20 mM phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



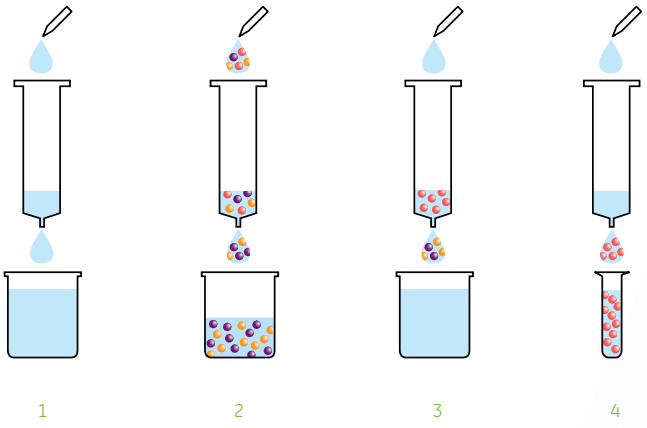
Summary

In this example, 50 mM imidazole prevented binding of most contaminants and improved purity (Lane 4). Adding more imidazole in the sample and binding buffer improved purity marginally but lowered yield (Lanes 5 and 6).

Manual purification at larger scale

Product featured: His GraviTrap

Direct purification, i.e. no clarification of the sample from bacteria cell lysates, can be performed by gravity methods using His GraviTrap columns. In this case, a high molecular weight (histidine)$_{10}$-tagged protein was purified in 20 min from 20 ml of clarified *E. coli* JM109 lysate containing (His)$_{10}$-TRX-P450 (M_r ~ 130 000).



1 Equilibrate
2 Load sample
3 Wash
4 Elute

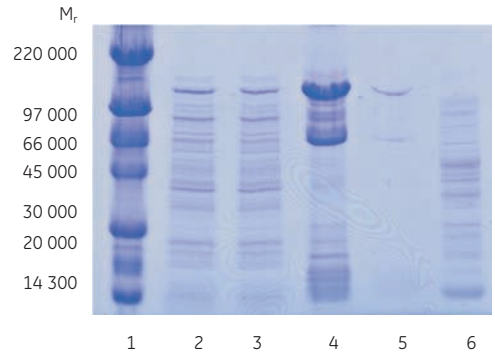
Total time: 20 min

Column: His GraviTrap

Sample: 20 ml clarified *E. coli* JM109 lysate containing (His)$_{10}$-TRX-P450 (M_r ~130 000)

Binding/wash buffer: 20 mM phosphate, 500 mM NaCl, 40 mM imidazole, pH 7.4

Elution buffer: 20 mM phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

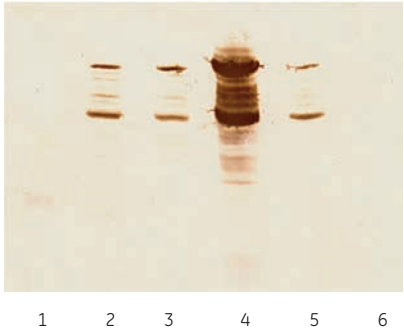


SDS-PAGE

(His)$_{10}$-TRX-P450

Lane

1 High-range rainbow molecular weight markers
2 Start material (diluted 1:20)
3 Flowthrough (diluted 1:20)
4 Eluate 1
5 Eluate 2
6 Negative control (JM109 non-transformed)



Western blot

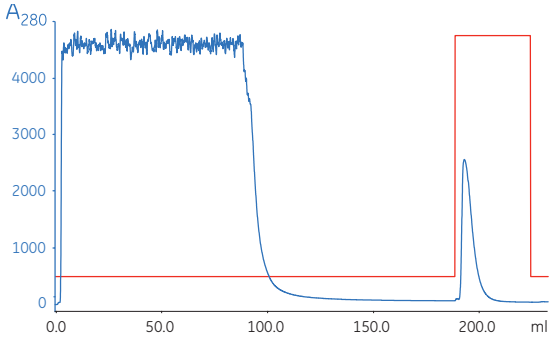
Summary

The purification took just 20 min. Both SDS-PAGE and Western blot analysis of the eluted fractions showed three major bands indicating that histidine tags were present. The top band is the targeted full-length protein and bands below are truncated forms of the histidine-tagged target protein.

Efficient two-step purification

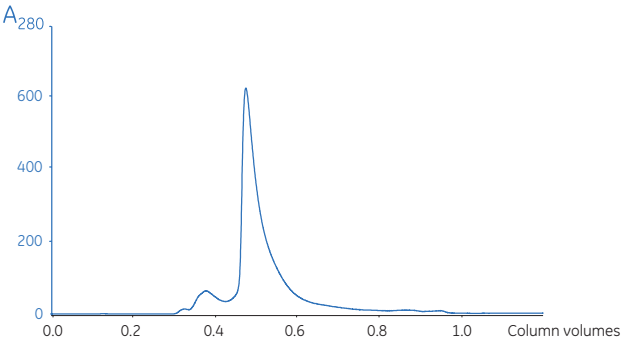
Products featured: HisTrap FF crude, HiLoad 26/60 Superdex 75 pg, ÄKTApurifier 10

Even when optimized conditions are used, one-step purification is not always enough to reach the required purity. Situations where this is the case include three-dimensional structural studies by X-ray crystallography, and when truncated forms of the target must be removed. For these situations a high degree of purity is required and intermediate and polishing steps can be necessary. The example below shows a two-step purification using ÄKTApurifier™.



Capture of histidine-tagged GFP in *E. coli* extract on HisTrap FF crude

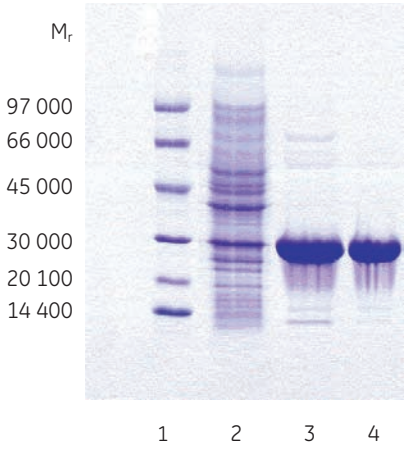
Column: HisTrap FF crude 5 ml
Sample: Histidine-tagged GFP (120 mg) in 86 ml *E. coli* extract
Flow rate: 5 ml/min
Binding and equilibration buffer: 20 mM sodium phosphate, 500 mM NaCl, 50 mM imidazole, pH 7.4
Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Equilibration: 5 column volumes (CV), 25 ml binding buffer
Wash: 20 CV binding buffer
Elution: 7 CV, one-step gradient elution buffer
System: ÄKTApurifier 10



Polishing of histidine-tagged GFP eluted from HisTrap FF crude on HiLoad 26/60 Superdex 75 pg

Column: HiLoad 26/60 Superdex 75 pg
Sample: 3 ml, eluted from HisTrap FF crude
Flow rate: 4 ml/min
Buffer: 50 mM phosphate buffer, 150 mM NaCl, pH 7.2

SDS-PAGE



Lane

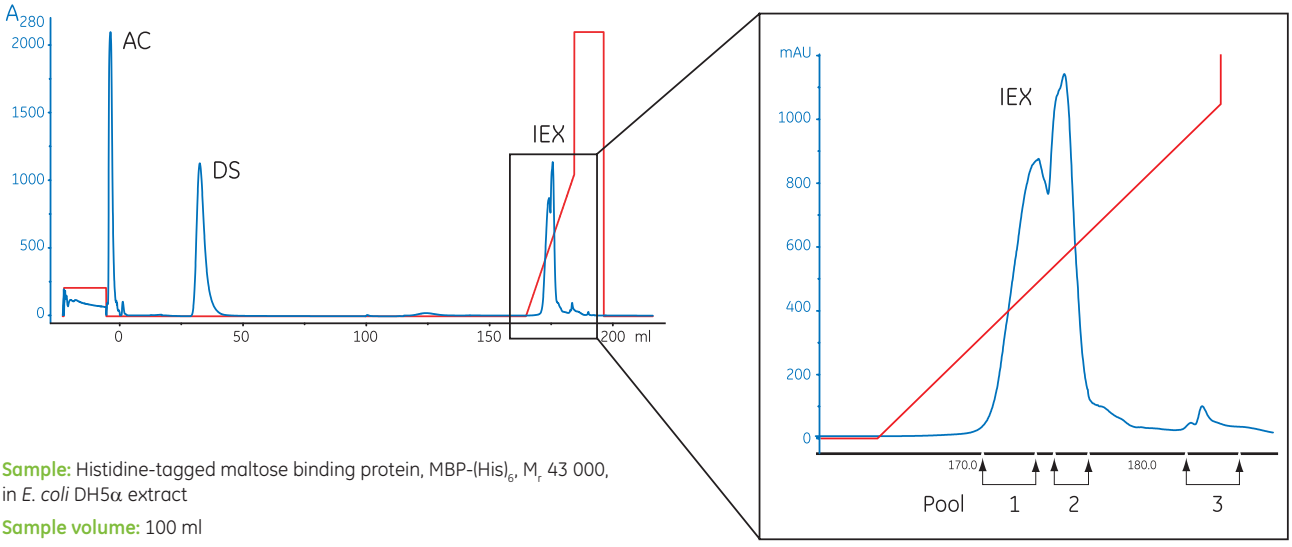
1 Low molecular weight markers
2 Start material
3 Capture step, eluted pool from HisTrap FF crude
4 Polishing step, pool from HiLoad 26/60 Superdex 75 pg

Summary
Gel filtration is a separation technique well suited to separating target protein from remaining impurities such as dimers yielding pure monomeric form for work such as structural determination.

Unattended three-step purification

Products featured: HisTrap FF crude, HiPrep 26/10 Desalting, Mono Q 5/50 GL, ÄKTAxpress

ÄKTAxpress™ with an automated three-step protocol was used to purify histidine-tagged maltose binding protein from 100 ml of an unclarified *E. coli* cell lysate. The three steps were: affinity chromatography (AC) using HisTrap FF crude (1 ml), desalting (DS) using HiPrep™ 26/10 Desalting, and ion exchange chromatography (IEX) using Mono Q™ 5/50 GL. These are referred to as AC/DS/IEX in the images.



AC/DS/IEX with an enlargement of the IEX peaks and the collected pools to the right. Yield: 9.4 mg in pools 1 + 2.

Sample: Histidine-tagged maltose binding protein, MBP-$(His)_6$, M_r 43 000, in *E. coli* DH5α extract

Sample volume: 100 ml

Columns: AC – HisTrap FF crude 1 ml. DS – HiPrep 26/10 Desalting. IEX – Mono Q 5/50 GL

AC binding buffer: 50 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, pH 8.0

AC elution buffer: 50 mM Tris-HCl, 0.5 M NaCl, 500 mM imidazole, pH 8.0

DS/IEX binding buffer: 50 mM Tris-HCl, pH 8.0

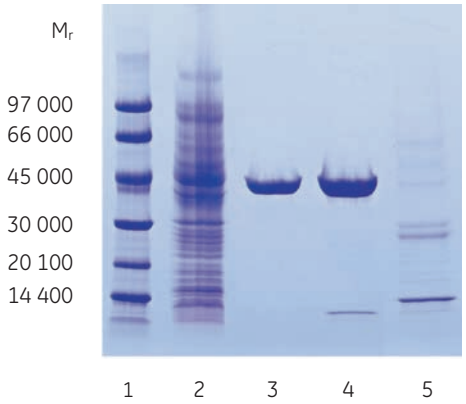
IEX elution buffer: 50 mM Tris-HCl, 1.0 M NaCl, pH 8.0

System: ÄKTAxpress™

SDS-PAGE

Lane

1 Low molecular weight markers
2 Start material, 1:10 diluted
3 Eluted pool 1 from IEX
4 Eluted pool 2 from IEX
5 Eluted pool 3 from IEX



Acknowledgements: GFP-$(His)_6$ was provided by Dr. David Drew, Dept. of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden. MBP-$(His)_6$ was provided by Phadia, Uppsala, Sweden.

Summary

As seen from the SDS-PAGE analysis, the purification gave a highly concentrated pure protein.

Unattended two-step purification

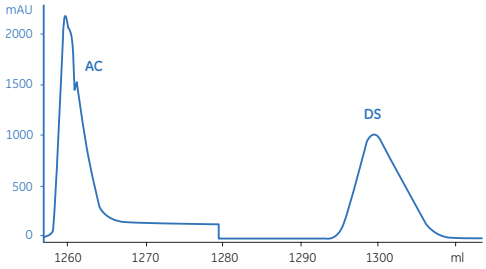
Products featured: HisTrap HP, HiPrep 26/10 Desalting, ÄKTAxpress

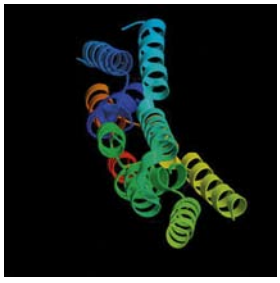
When structural determinations are automated, the supply of pure proteins can be bottlenecked. ÄKTAxpress provides a complete, intelligent, and robust solution for unattended multistep purification of affinity-tagged proteins and antibodies. The system automatically runs multiple samples and eliminates time-consuming manual tasks during protein purification for structural and functional studies.

ÄKTAxpress was successfully used to purify proteins for 3-D structural determinations. Here are some examples:

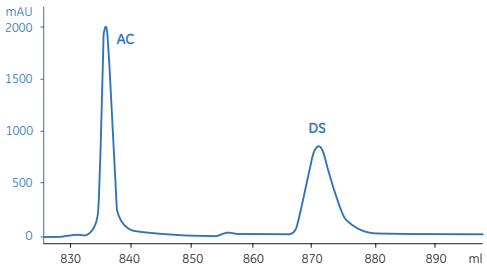
AC = Affinity chromatography
DS = Desalting

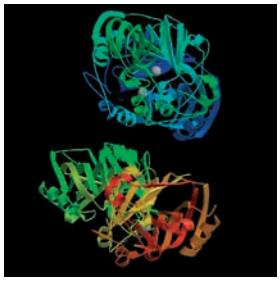
APC298 (129 aa)
pI: 6.74
M_r: 17 120
Hypothetical protein:
NP_646141.1 domain 3912-4037
Source: *Staphylococcus aureus subsp. aureus MW2*



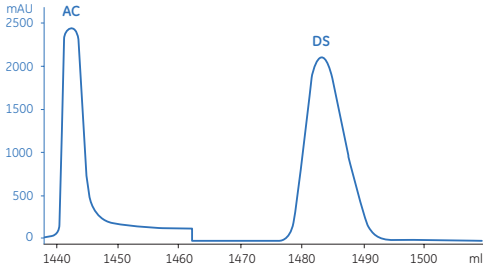


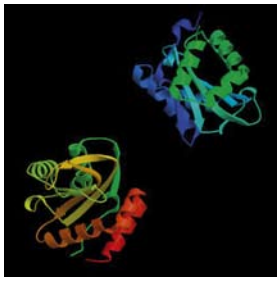
APC23686 (234 aa)
Hypothetical protein: Similar to alpha-acetolactate decarboxylase
Source: *Staphylococcus aureus subsp. aureus N315*





APC25506 (157 aa)
pI: 4.71
M_r: 20 580
Hypothetical protein: Putative phosphotransferase system mannitol/ fructose-specific IIA domain
Source: *Salmonella typhimurium LT2.*

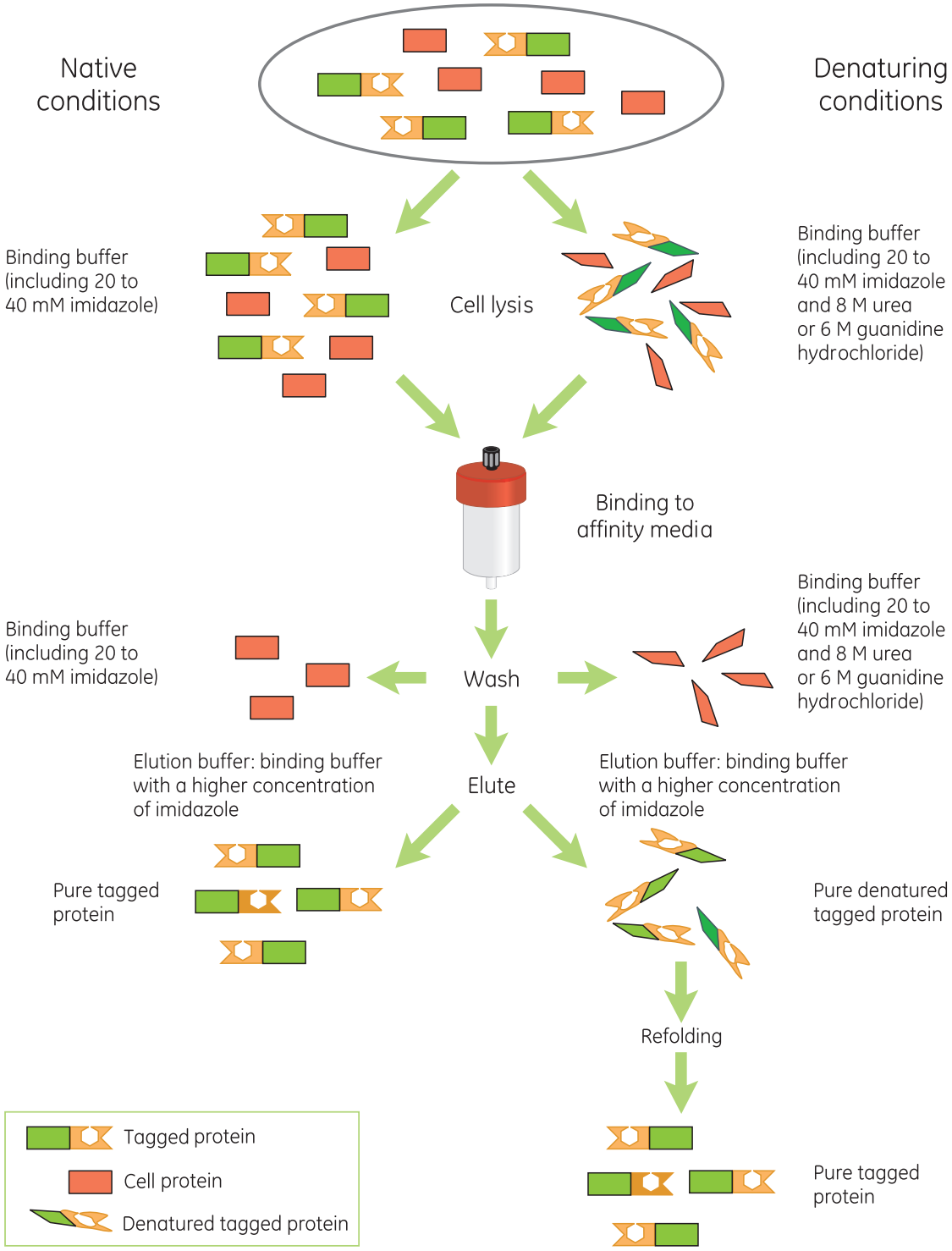




Acknowledgement: A. Joachimiak, Y. Kim, M. Zhou, Structural Biology Center & Midwest Center for Structural Genomics, Biosciences Division, Argonne National Laboratory.

Summary
Using automated optimized protocols minimizes hands-on time, while ensuring throughput of highly pure proteins for structural determination.

General purification scheme for histidine-tagged proteins



GST-tagged proteins

High purity in one simple step

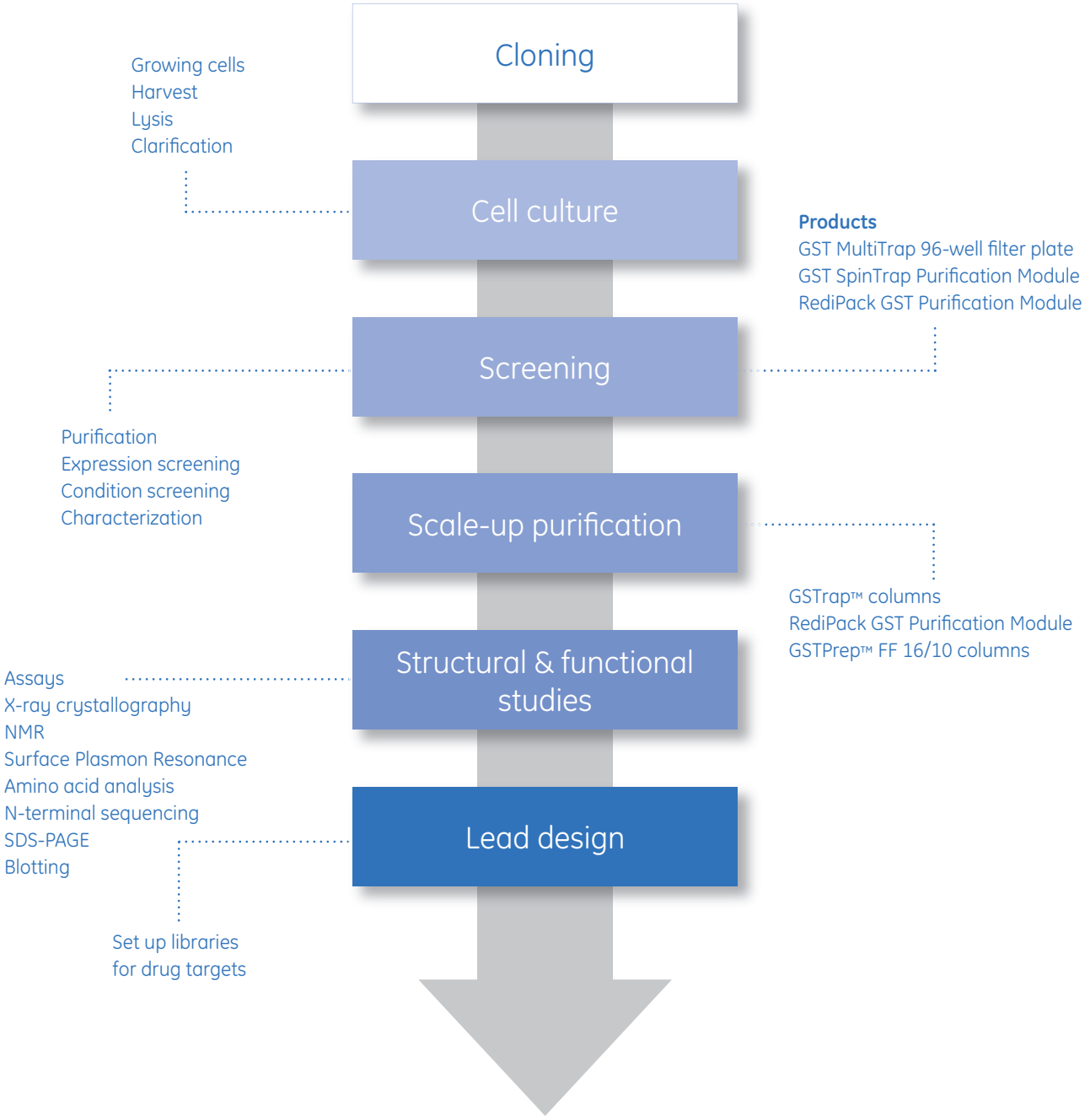
Glutathione S-transferase (GST) Gene Fusion System is a versatile system for expression, purification, and detection of GST-tagged proteins produced in *E. coli*. Typical features of GST-tagged proteins include high binding specificity to glutathione ligands on Glutathione Sepharose, resulting in very high purity of eluted target molecule. The tag is relatively large (M_r 26 000). A specific cleavage sequence allows simple removal of the tag after purification. It may also increase the solubility and stability of the protein.

Purification of GST-tagged proteins can be done under very mild conditions, which preserves the function and antigenicity of the target protein. GST tags are often used to complement histidine tags or as an alternative when histidine tags do not give a soluble protein during expression.

Cleavage
Removal of the GST tag is often necessary for studies of target proteins. PreScission™ Protease offers an efficient method for specific cleaving and removing GST tags. The optimum working temperature is 4°C, which makes PreScission Protease useful when working with labile and sensitive proteins.

Cleavage can also be accomplished using other proteases that recognize different cleavage sites. Factor Xa and thrombin are serine proteases with optimal cleavage performance at room temperature.

From gene to target protein



The workflow above is a typical example of a protein production process that starts at the gene and ends with a target protein. To simplify your lab work, we offer a wide range of products that fit into your workflow.

High-throughput screening of lysis methods

Product featured: GST MultiTrap 4B

Efficient and rapid screening is an essential step in the study of protein expression, function, and structure of recombinant proteins. With GST MultiTrap 4B 96-well filter plates, unclarified sample can be applied directly to keep handling to a minimum for the best possible recovery. The plates are prepacked with media for high-throughput parallel screening of GST-tagged proteins. Different lysis methods – CelLytic™ Express, BugBuster™ Protein Extraction Reagent, sonication, and enzymatic lysis – were used to investigate the difference in yield and reproducibility.

	CelLytic Express	BugBuster	Sonication	Enzymatic lysis
Average yield of eluted protein (µg)	361	287	264	261
Standard deviation (µg)	38	27	31	20
Relative standard deviation (%)	11	9	12	7

Screening method

96-well filter plate: GST MultiTrap 4B

Handling: Centrifugation

Sample: *E. coli* expression GST-hippocalcin (M_r 45 000)

Sample volume: 500 µl

Binding/wash buffer: 10 mM sodium phosphate, 140 mM NaCl, pH 7.4

Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

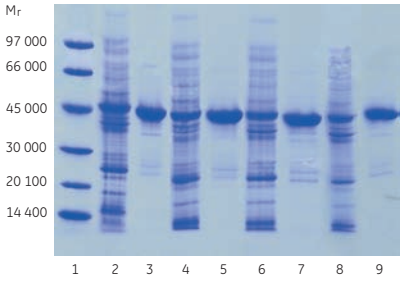
Elution method: Centrifugation

Data evaluation: UV spectrometry (A_{280}) and SDS-PAGE

SDS-PAGE

Lane

1 Low molecular weight markers
2 Start material, CelLytic Express (diluted 1:20)
3 Eluted pool, CelLytic Express (diluted 1:5)
4 Start material, BugBuster (diluted 1:20)
5 Eluted pool, BugBuster (diluted 1:5)
6 Start material, sonication (diluted 1:20)
7 Eluted pool, sonication (diluted 1:5)
8 Start material, enzymatic lysis (diluted 1:20)
9 Eluted pool, enzymatic lysis (diluted 1:5)



Summary

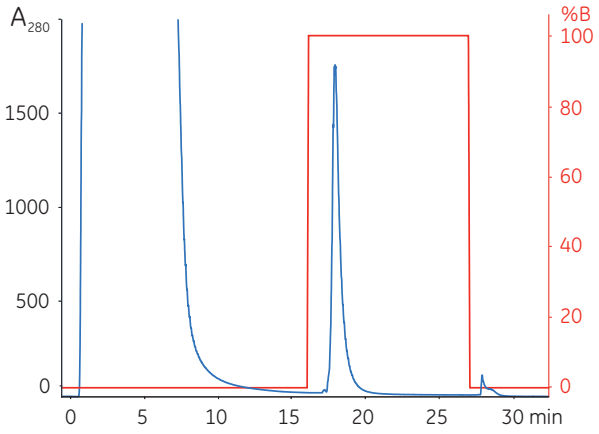
High yield and purity were achieved with all four lysis methods.

Simple two-step purification

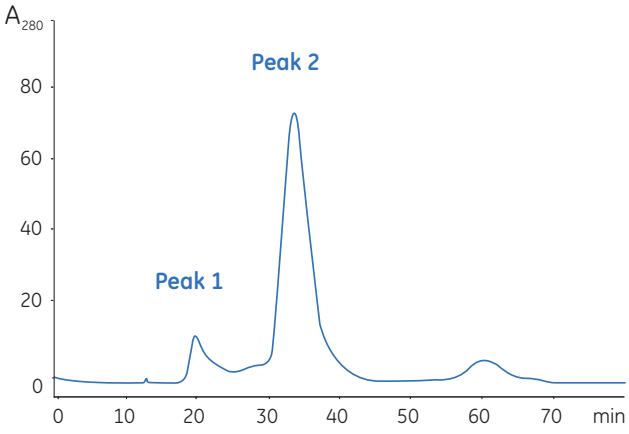
Products featured: GSTrap 4B, HiLoad 16/60 Superdex 200 pg, ÄKTAprime plus

High purity can be achieved with a simple chromatography system. Using ÄKTAprime™ plus, GST-hippocalcin was purified in two steps with a GSTrap 4B column, followed by gel filtration with a HiLoad 16/60 Superdex 200 pg column. ÄKTAprime plus has preprogrammed methods and optimized protocols that simplify the purification tasks.

Capture



Polishing



Columns: Capture – GSTrap 4B 1 ml
Polishing – HiLoad 16/60 Superdex 200 pg

Sample: Cell extract from *E. coli* expressing GST-hippocalcin (M_r 45 000) after lysis with CelLytic Express

AC binding/wash buffer: 10 mM sodium phosphate, 140 mM, pH 7.4

AC elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

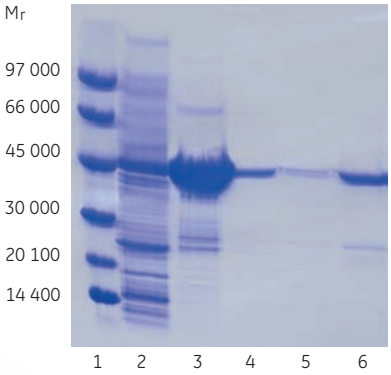
GF buffer: 50 mM phosphate buffer, 150 mM NaCl, pH 7.2

System: ÄKTAprime plus

SDS-PAGE, Coomassie™ staining

Lane

1 Low molecular weight markers
2 Start material (diluted 1:20)
3 Eluted pool GSTrap 4B
4 Eluted pool GSTrap 4B (diluted 1:50)
5 Peak 1, HiLoad 16/60 Superdex 200 pg
6 Peak 2, HiLoad 16/60 Superdex 200 pg



Summary
Addition of a second purification step using polishing with gel filtration increased the target protein purity.

Automated multistep purification and tag removal

Products featured: GSTrap HP, HiLoad 16/60 Superdex 75 pg, ÄKTAxpress

It is often necessary to remove the large GST tag from the purified target protein. GST-tagged proteins produced with a PreScission Protease cleavage site enable single-step purification with on-column tag cleavage. A GST-tagged model protein was purified using ÄKTAxpress automated two-step purification method. GST tag cleavage and removal were also incorporated in the protocol.

Columns: Capture – Affinity Chromatography (AC), GSTrap HP 5 ml
Polishing – Gel Filtration (GF), HiLoad 16/60 Superdex 75 pg

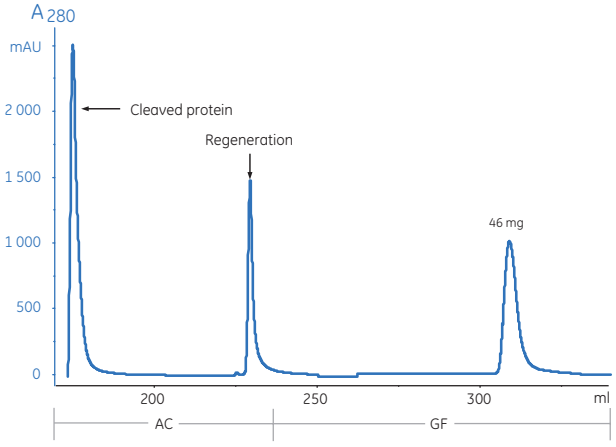
Sample: GST-purα (M_r 61 600)

AC binding/cleavage buffer: 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5

AC elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

GF buffer: 50 mM Tris-HCl, 150 mM NaCl, pH 7.5

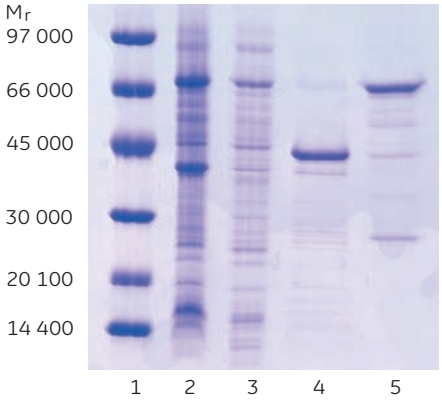
System: ÄKTAxpress



SDS-PAGE

Lane

1	Low molecular weight markers
2	Start sample GST-purα
3	Flowthrough
4	Purified, cleaved GST-purα (M_r 35 200) after AC-GF
5	Reference uncleaved GST-purα (M_r 61 600)

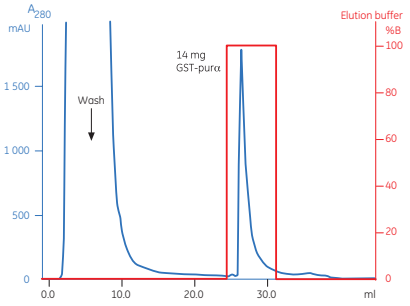


Summary
Tag cleavage and removal were incorporated in the automated two-step purification protocol. This resulted in pure target protein with the tag cleaved off.

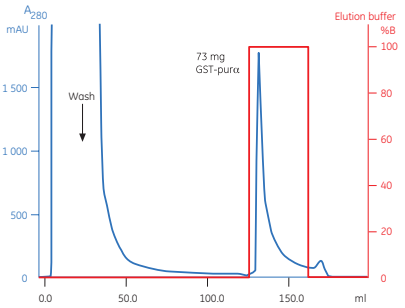
Increasing the purification scale

Products featured: GSTrap FF, GSTPrep FF 16/10, ÄKTAexplorer 100

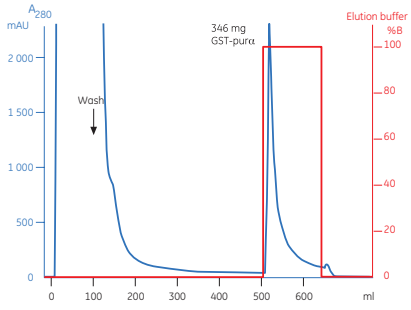
Once a method for the purification of a target protein has been developed, it can be scaled up to produce larger quantities of target protein for further studies and assays. The one-step purification method below illustrates a 26-fold scale-up. The main parameter in this scale-up study was residence time (the period of time the sample was in contact with the chromatography medium). Residence time was the same for the GSTrap FF 1 ml and 5 ml columns, but twice as long for the GSTPrep FF 16/10 (20 ml column) due to the difference in column length and diameter.



GSTrap FF 1 ml



GSTrap FF 5 ml



GSTPrep FF 16/10

Columns: GSTrap FF 1 ml, GSTrap FF 5 ml, GSTPrep FF 16/10

Samples: 5 ml, 25 ml, and 100 ml *E. coli* extract expressing GST-purα

Binding buffer: PBS, pH 7.4

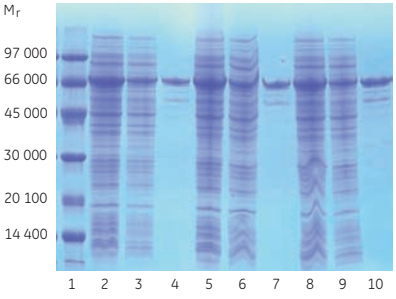
Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

System: ÄKTAexplorer 100

SDS-PAGE

Lane

1 Low molecular weight markers
2 Extract, 1 g *E. coli* cells/5 ml
3 Flowthrough from GSTrap FF 1 ml
4 GST-purα eluted from GSTrap FF 1 ml
5 *E. coli* expressing GST-purα, 1 g cell paste/5 ml
6 Flowthrough from GSTrap FF 5 ml
7 GST-purα eluted from GSTrap FF 5 ml
8 *E. coli* expressing GST-purα, 1 g cell paste/5 ml
9 Flowthrough from GSTPrep FF 16/10
10 GST-purα eluted from GSTPrep FF 16/10



Summary
The amount of eluted GST-tagged protein increased proportionally with increased column volume and sample load in the scale-up protocols.

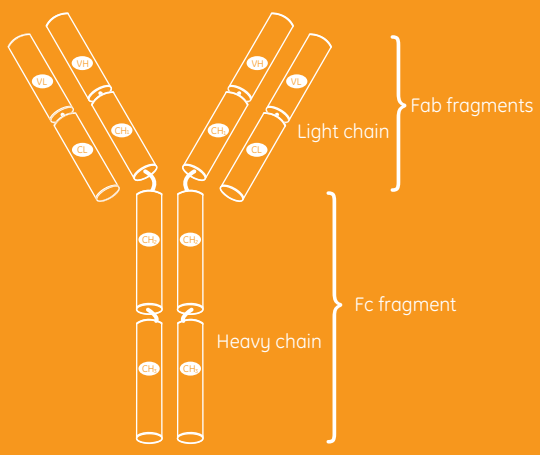
Antibody purification

Flexible solutions to meet your goals

There are growing numbers of research, therapeutic, and diagnostic applications for monoclonal antibodies (MAbs), polyclonal antibodies, and their fragments. The high specificity of Protein A and Protein G for the Fc region of antibodies is a significant advantage for achieving efficient purification. Polyclonal antibodies are commonly used as reagents in immunochemical techniques, using crude serum as a source. Despite high purity in one step (> 95% purity) using Protein A or Protein G ligands, a polishing step using gel filtration is usually required to remove aggregates and/or dimers.

Relative binding strengths

Protein A Sepharose and Protein G Sepharose affinity media have different specificities for IgG. Protein G Sepharose media are the better choice for general purpose capture of antibodies since they bind IgG from a broader range of eukaryotic species and bind more subclasses of IgG. Protein A Sepharose media may also be used for capture of IgA, and can be the better choice for isolating certain cross-species IgG, such as contaminants from horse or fetal calf serum. The relative binding strengths of polyclonal IgG from various species and subclasses to Protein A and Protein G are listed in the specificity table as measured in a competitive ELISA test.



Species	Subclass	Protein A	Protein G
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	IgM*	+++	+++
Rabbit	–	++++	+++
Rat	IgG_1	–	+
	IgG_{2a}	–	++++
	IgG_{2b}	–	++
	IgG_3	+	++
Sheep	IgG_3	+/–	++

* Purify using HiTrap IgM Purification HP columns
– Weak or no binding
++ Medium binding
++++ Strong binding
×× Purify using HiTrap IgY Purification HP columns

Purification of unclarified anti-HSA serum

Product featured: Ab SpinTrap

Purification of antibodies from serum without sample clarification, dilution or filtration is possible with Ab SpinTrap columns. In this example, undiluted serum from an immunized rabbit was applied to the column and the purification steps performed using a microcentrifuge.

Column: Ab SpinTrap, prepacked with 100 µl Protein G Sepharose High Performance

Equilibration: 600 µl 20 mM sodium phosphate, pH 7.0

Sample: 600 µl undiluted serum

Incubation: 4 min

Wash: 2 × 600 µl 20 mM sodium phosphate, pH 7.0

Elution: 2 × 400 µl 0.1 M glycine-HCl, pH 2.7

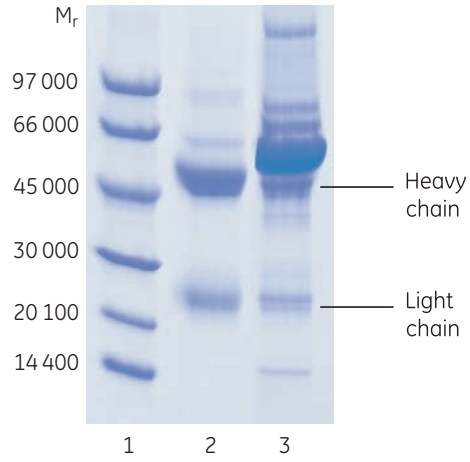
SDS-PAGE

Lane

1 Low molecular weight markers

2 Eluted pool (diluted 1:5)

3 Start material (diluted 1:50)



Summary

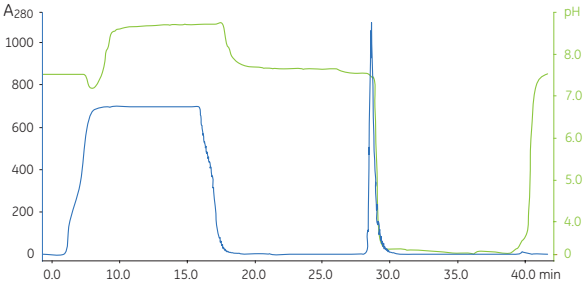
The purification yielded 2 mg of antibody with more than 90% purity according to SDS-PAGE under reducing conditions.

Purification of monoclonal mouse IgG_1

Products featured: HiTrap Protein G HP, HiLoad 16/60 Superdex 200 pg, ÄKTAprime plus

This case shows a generic purification method for monoclonal IgG antibodies from cell culture supernatant using ÄKTAprime plus. It is based on a capture step using affinity chromatography, followed by a polishing step using gel filtration. Monoclonal mouse IgG_1 was captured in the first step and eluted using a low pH buffer.

Capture

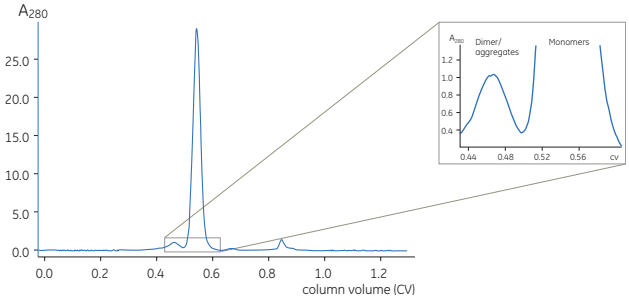


Curves from the capture step on HiTrap™ Protein G HP

Capture

Column: HiTrap Protein G HP 1 ml
Equilibration: 5 column volumes (CV), 20 mM sodium phosphate, pH 7.0
Sample: 10 ml cell culture supernatant containing monoclonal mouse IgG_1
Wash: 10 CV 20 mM sodium phosphate, pH 7.0
Elution: 10 CV 0.1 M glycine-HCl, pH 2.7
Re-equilibration: 5 CV 20 mM sodium phosphate, pH 7.0
Flow rate: 1 ml/min
System: ÄKTAprime plus

Polishing



Note the separation between dimers and monomers (magnified)

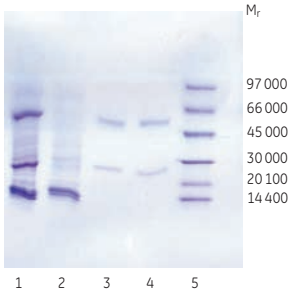
Polishing

Column: HiLoad 16/60 Superdex 200 pg
Buffer: 50 mM phosphate and 150 mM sodium phosphate, pH 7.2
Sample: Pooled fractions from the capture step, 2 ml
Flow rate: 1 ml/min
System: ÄKTAprime plus

SDS-PAGE

Lane

1 Monoclonal mouse IgG_1 (cell culture supernatant)
2 Flowthrough (capture step)
3 Eluted fractions (capture step)
4 Eluted fractions (polishing step)
5 Low molecular weight markers



Summary

Purity was controlled by SDS-PAGE under reducing conditions, which showed that the antibody was highly pure after the first affinity step. The gel filtration step further improved target quality by separating the dimer and monomer of the antibody. Note that the dimers run as monomers in reducing SDS-PAGE.

Unattended two-step purification of antibodies

Products featured: HiTrap MabSelect SuRe, HiPrep 26/10 Desalting, ÄKTAxpress

In this application ÄKTAxpress was used for automated two-step purification of antibodies at milligram scale. One- and two-step protocols including cleaning-in-place (CIP) procedures can be easily generated by the method wizard. This example demonstrates automated two-step purification.

Protocol: Automated two-step purification, affinity chromatography (AC) and desalting (DS)

Columns: HiTrap MabSelect SuRe™ 1 ml and HiPrep 26/10 Desalting

Sample: 20 ml human monoclonal antibody in culture supernatant, ~0.5 mg/ml

Sample volume: 20 ml

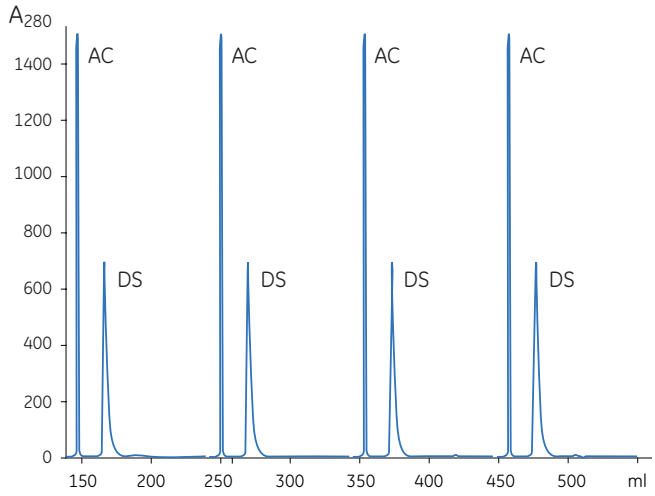
Affinity binding buffer: 20 mM phosphate, 150 mM NaCl, pH 7.0

Affinity elution buffer: 100 mM sodium citrate, pH 3.0

Desalting buffer: 50 mM phosphate buffer, 150 mM NaCl, pH 7.2

Flow rate: 1 ml/min

System: ÄKTAxpress

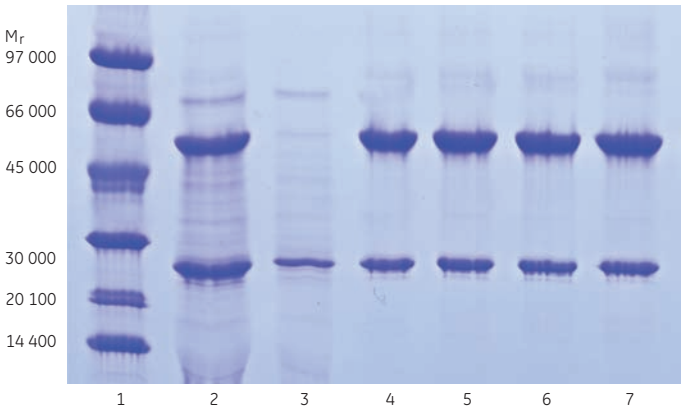


Chromatogram of four repetitive runs showing purification of human monoclonal antibody from cell culture by affinity and desalting.

SDS-PAGE

Lane

1 Low molecular weight markers
2 Start material
3 Flowthrough
4 Run 1
5 Run 2
6 Run 3
7 Run 4



Lanes 4-7 are pooled fractions from the final desalting steps

Acknowledgment: G.J. Perdock, T. Verhagen and P.H.C. van Berkel, Genmab BV, Utrecht, Netherlands

Summary
The desalting step is important for the preservation of physiological conditions and activity. On average, 8.3 mg (+/- 0.17 mg) of highly pure target antibody was achieved after the automated two-step purification.

Purification of monoclonal α-Shigella IgM

Products featured: HiTrap IgM Purification HP, ÄKTApurifier 10

This application is optimized for purification of monoclonal α-Shigella IgM from hybridoma cell culture. It can also be used as a starting point to determine the binding and elution conditions required for other IgM preparations such as human IgM.

Sample: 75 ml of cell culture supernatant containing α-Shigella IgM

Column: HiTrap IgM Purification HP

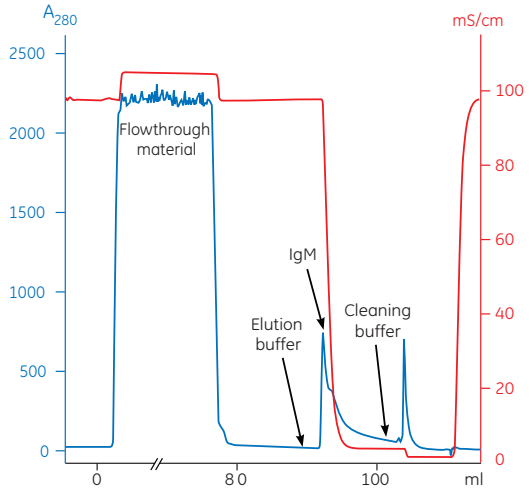
Binding buffer: 20 mM sodium phosphate buffer, 0.5 M potassium sulfate, pH 7.5

Elution buffer: 20 mM sodium phosphate buffer, pH 7.5

Cleaning buffer: 20 mM sodium phosphate buffer, pH 7.5, 30% isopropanol

Flow rate: 1 ml/min

System: ÄKTApurifier 10

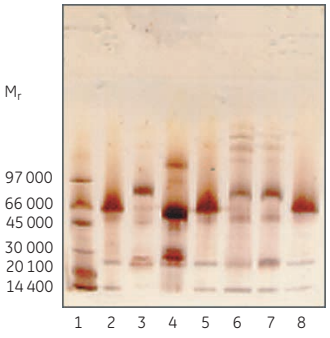


SDS-PAGE, silver stained

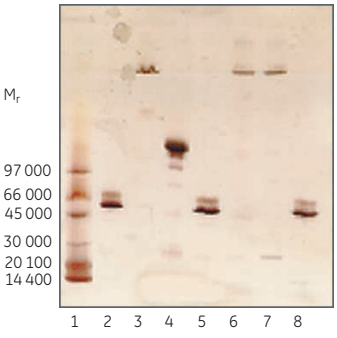
Lane

1 Low molecular weight markers
2 Start material, cell culture supernatant diluted 1:20
3 Reference sample: IgM, human
4 Reference sample: IgG
5 Flowthrough pool, diluted 1:20
6 Eluted IgM, fraction 8, diluted 1:8
7 Eluted IgM, fraction 9, diluted 1:8
8 Washing out unbound material, pool diluted 1:3

Samples reduced with 2-mercaptoethanol



Non-reduced samples



Summary

SDS-PAGE analysis demonstrated a purity level of over 80% after one simple purification step using HiTrap IgM Purification HP, and results from an ELISA (not shown) indicated high activity in the purified fraction.

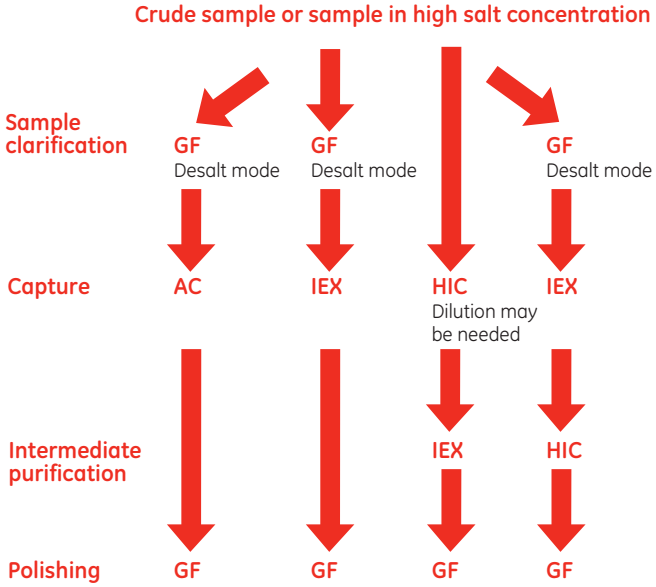
Purifying native and untagged recombinant proteins

All protein purification has a common goal: to purify enough of the active product without loss of target protein, time or money. For native or untagged recombinant proteins, purification strategies can vary from simple single-step procedures to complex validated processes for biopharmaceutical manufacture.

Purification challenges
Successful protein purification frequently requires a multistep approach: capture, intermediate purification, and polishing (CiPP). Specific objectives are assigned to each step:

> Capture: isolates, concentrates, and stabilizes the target protein
> Intermediate purification: removes most bulk impurities, such as other proteins and nucleic acids, endotoxins, and viruses
> Polishing: achieves high purity by removing any remaining trace impurities or closely related substances

Solutions
Our wide range of lab-scale media and prepacked columns for purifying proteins cover all major chromatographic techniques and allow you to:
> Choose logical combinations of purification techniques based on the main benefits of the technique and the condition of the sample at the beginning and end of each step
> Reduce sample handling between purification steps by combining the best techniques
> Increase yield while saving time and money by using as few steps as possible
> If you are not sure which purification strategy to use, employ an effective general approach: IEX (capture), HIC (intermediate purification) and GF (polishing)



AC: Affinity chromatography

IEX: Ion exchange chromatography

GF: Gel filtration

HIC: Hydrophobic interaction chromatography

RPC: Reversed phase chromatography

Technique	Main features	Capture	Intermediate	Polishing	Sample start condition	Sample end condition
IEX	High resolution High capacity High speed	★★★	★★★	★★★	Low ionic strength, sample volume not limiting	High ionic strength or pH change, concentrated
HIC	Good resolution Good capacity High speed	★★	★★★	★	High ionic strength, sample volume not limiting	Low ionic strength, concentrated
AC	High resolution High capacity High speed	★★★	★★★	★★	Specific binding conditions, sample volume not limiting	Specific elution conditions, concentrated
GF	High resolution using Superdex		★	★★★	Limited sample volume (< 5% total column volume) and flow rate range	Buffer exchanged (if required), diluted
RPC	High resolution		★	★★★	Requires organic solvents	Risk loss of biological activity, concentrated

Three-step purification for crystallization

Products featured: HiTrap Capto Q, HiTrap Butyl FF, HiLoad 16/60 Superdex 200 pg, ÄKTAexplorer 10

This example shows how a three-step purification of subunit R2 of ribonucleotide reductase is performed using ÄKTAexplorer prior to crystallization. The protein originates from *M. tuberculosis* and was overexpressed intracellularly in *E. coli*. The target absorbs light at both 280 nm and 408 nm, which allowed efficient identification, quantitation, and calculation of purity in the chromatographic steps, which were:

1. Capture by anion exchange chromatography using HiTrap Capto™ Q
2. Intermediate purification by hydrophobic interaction chromatography using HiTrap Butyl FF
3. Polishing by gel filtration using HiLoad 16/60 Superdex 200 prep grade

1. Capture step

Column: HiTrap Capto Q 5 ml

Sample: 50 ml subunit R2 of ribonucleotide reductase

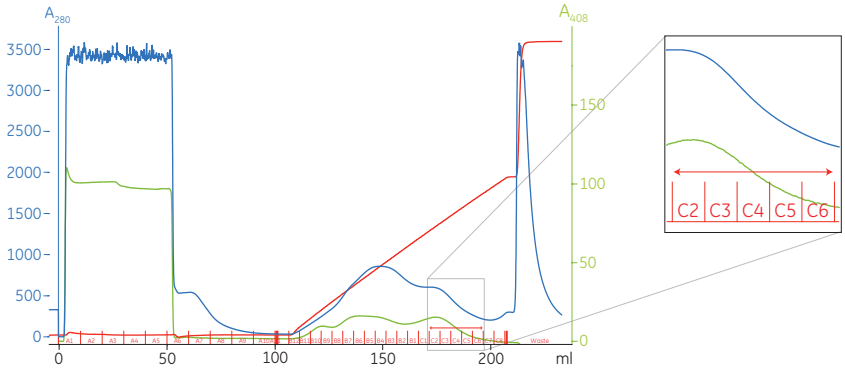
Flow rate: 4 ml/min

Start buffer: 20 mM Tris-HCl, 50 mM NaCl, pH 7.5

Elution buffer: 20 mM Tris-HCl, 1 M NaCl pH 7.5

Elution gradient: Linear 0-50% elution buffer 1000 ml (20 column volumes)

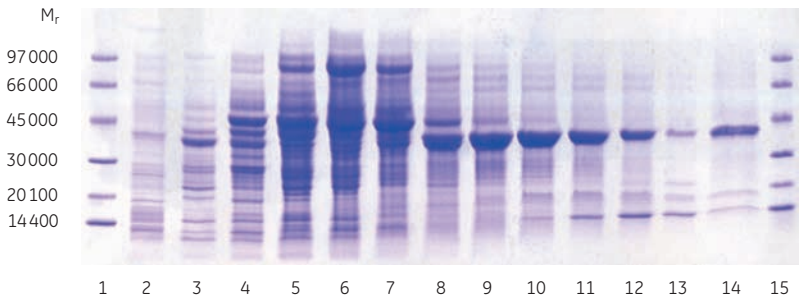
System: ÄKTAexplorer 10



Chromatogram showing the target protein is magnified. Fraction C2-C6 is purified further in step two.

SDS-PAGE

Lane	
1	Low molecular weight markers
2	Fraction A1–A5 diluted 10 times
3	Fraction B10
4	Fraction B8
5	Fraction B6
6	Fraction B4
7	Fraction B2
8	Fraction C1
9	Fraction C2
10	Fraction C3
11	Fraction C4
12	Fraction C5
13	Fraction C6
14	Fraction C2–C6 pooled
15	Low molecular weight markers



2. Intermediate step

Column: HiTrap Butyl FF 5 ml

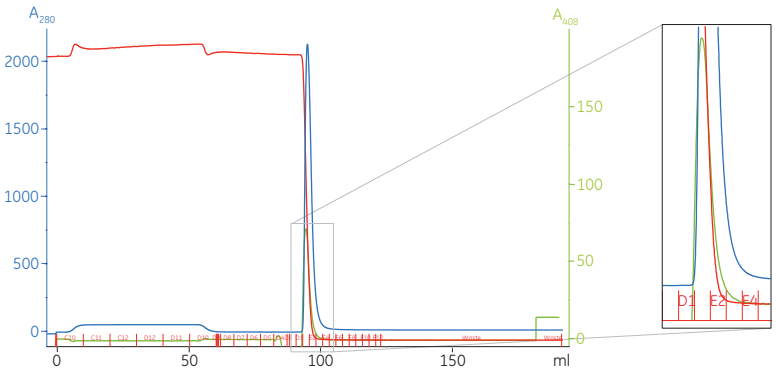
Sample: 50 ml pooled fractions from HiTrap Capto Q

Flow rate: 4 ml/min

Start buffer: 20 mM Tris-HCl, pH 7.5

Elution buffer: 20 mM Tris-HCl, 1.5 M ammonium sulfate, pH 7.5

Gradient: One step, 100% to 0% elution buffer



Chromatogram showing the target protein is magnified. Fractions from the main peak were pooled and purified further.

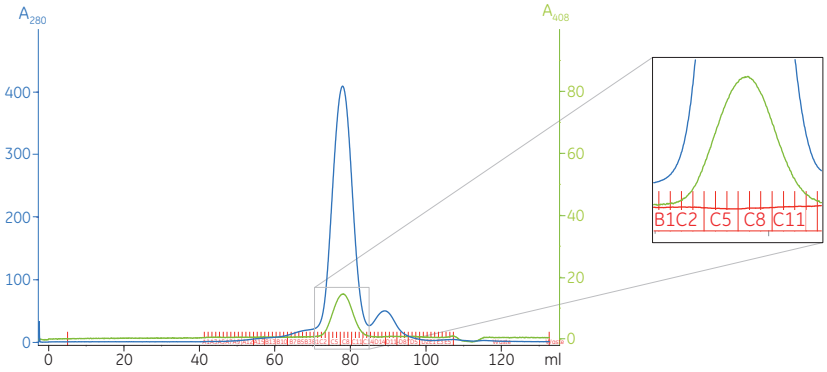
3. Polishing step

Column: HiLoad 16/60 Superdex 200 pg

Sample: 5 ml pooled fractions from HiTrap Butyl FF

Flow rate: 1 ml/min

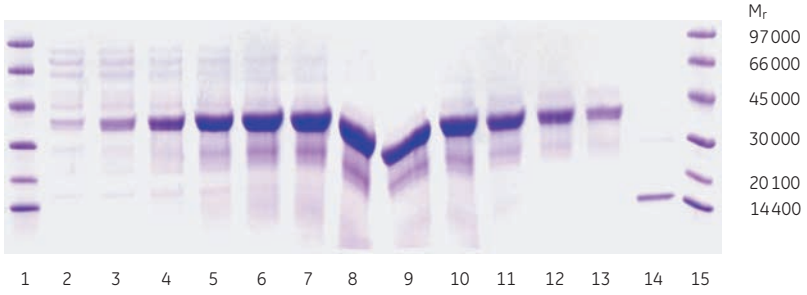
Buffer: 50 mM HEPES, 200 mM Na_2SO_4, pH 7.0

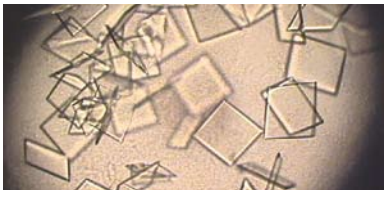


Chromatogram showing the target protein is magnified. Fraction C2-C13 and D13 were analyzed by SDS-PAGE.

SDS-PAGE

Lane	
1	Low molecular weight markers
2	Fraction C2
3	Fraction C3
4	Fraction C4
5	Fraction C5
6	Fraction C6
7	Fraction C7
8	Fraction C8
9	Fraction C9
10	Fraction C10
11	Fraction C11
12	Fraction C12
13	Fraction C13
14	Fraction D13
15	Low molecular weight markers





Crystals formed and allowed to grow for one month.

Acknowledgement: Prof. Torsten Unge, Institute of Cell and Molecular Biology, Uppsala University, Sweden.

Summary

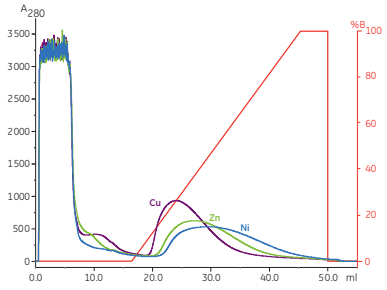
Since the protein is labile, the purification was optimized so that the entire purification, including cell lysis, chromatography, and SDS-PAGE analyses, was performed in one day. Crystallization of the purified protein was successful.

Purification of an untagged recombinant protein by IMAC

Products featured: HiTrap IMAC FF, ÄKTAexplorer 100

It is possible to purify native or untagged recombinant proteins having exposed histidine residues by immobilized metal ion affinity chromatography (IMAC). By using uncharged IMAC Sepharose 6 Fast Flow, the user can immobilize a suitable metal ion to capture the target protein and optimize conditions. In this case, recombinant bovine carbonic anhydrase II (r-BCA) is used as an example for selection of the most suitable metal ion and elution conditions for scale-up using ÄKTAexplorer.

Selection of metal ions for capture



Based on the elution positions of the bound proteins, the affinity of the metal ions for r-BCA was in the order Zn^{2+} = Ni^{2+}> Cu^{2+}.

Column: HiTrap IMAC FF 1 ml

Metal ions: Zn^{2+}, Ni^{2+}, or Cu^{2+}

Sample: 5 ml clarified *E. coli* extract containing 30 mg of r-BCA

Flow rate: 1 ml/min

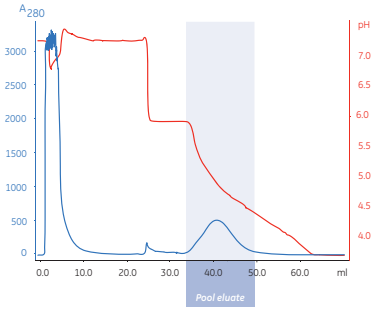
Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4

Elution: Linear gradient (30 CV) from 0% to 100% elution buffer

System: ÄKTAexplorer 100

Chosen metal ion with pH gradient elution



Purification of r-BCA on a HiTrap IMAC FF, 1 ml charged with Zn^{2+}. The bound fraction was eluted with a linear pH gradient.

Column: HiTrap IMAC FF 1 ml charged with Zn^{2+}

Sample: 2.5 ml clarified *E. coli* extract containing 15 mg r-BCA

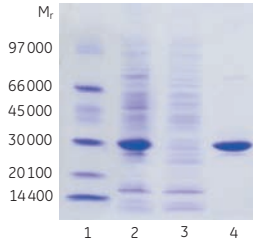
Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, pH 7.4

Elution buffer 1: 20 mM sodium acetate, 500 mM NaCl, pH 6.0

Elution buffer 2: 20 mM sodium acetate, 500 mM NaCl, pH 4.0

Flow rate: 1 ml/min

Elution: Stepwise elution in 15 CV to pH 6 (elution buffer 1) followed by a linear gradient from pH 6 to pH 4



SDS-PAGE

Lane	
1	Low molecular weight markers
2	Clarified *E. coli* extract
3	Flowthrough
4	Eluted pool

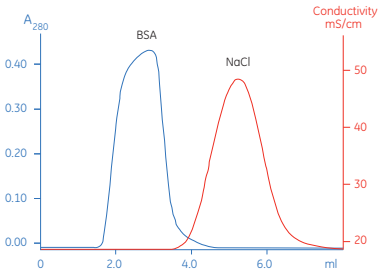
Summary

The Zn^{2+} ion was selected for immobilization since it is less toxic and more environmentally friendly than other ions used in this study. The target was successfully purified in one step using a pH gradient. Elution with a pH gradient instead of imidazole buffer elution also makes this method more biocompatible and lowers the cost per run in larger-scale operations.

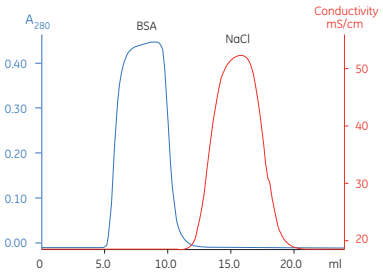
Desalting and buffer exchange

Products featured: HiTrap Desalting, ÄKTAFPLC

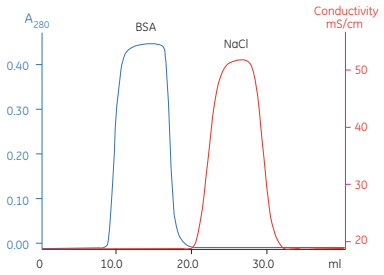
Buffer exchange and desalting are common sample preparation steps used in purification schemes. If you need to desalt large sample volumes it is simple to scale up by connecting up to five HiTrap Desalting columns in series. For larger volumes, HiPrep Desalting is recommended.



HiTrap Desalting 1 × 5 ml



HiTrap Desalting 3 × 5 ml in series



HiTrap Desalting 5 × 5 ml in series

Columns: HiTrap Desalting (Sephadex™ G-25 Superfine), 1 × 5 ml, 3 × 5 ml, 5 × 5 ml in series

Sample: 2 mg/ml BSA in 50 mM sodium phosphate, 0.5 M NaCl, pH 7.0

Sample volume: 28% of column volume (1.4, 4.3 and 7.1 ml respectively)

Buffer: 50 mM sodium phosphate, 0.15 M NaCl, pH 7.0

Flow rate: 5 ml/min

System: ÄKTAFPLC™

Summary
The results show that one, three and five HiTrap Desalting columns in series can desalt sample volumes up to 1.4, 4.3, and 7.1 ml. Connecting the columns in series allowed fast and simple scale-up.

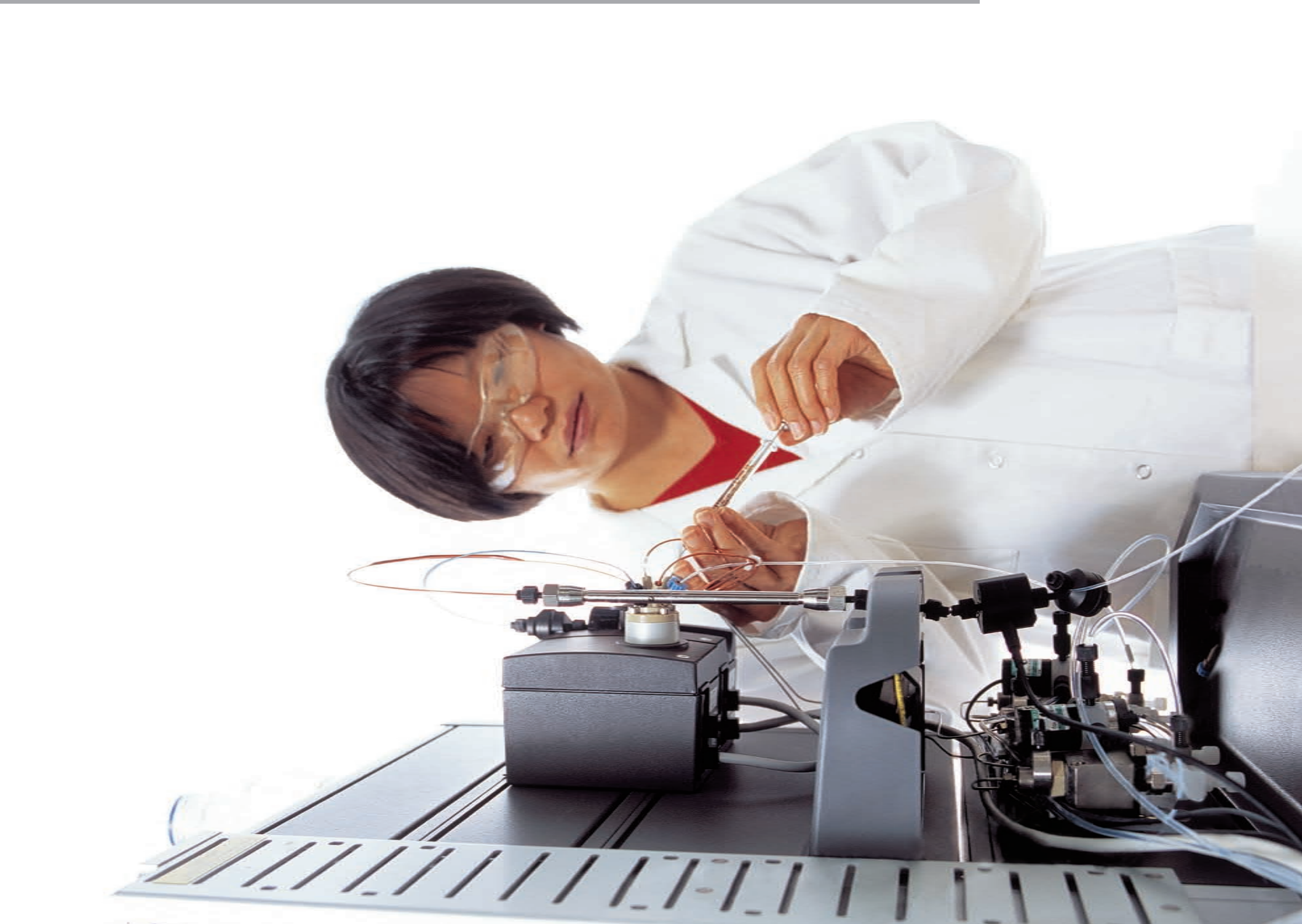
Analytical separations

Comprehensive solutions for complex challenges

In protein purification it is vital to find techniques for the identification and characterization of impurities and the target molecule. Common analysis tasks include:

> Purity check
> Quantitation
> Analysis of post-translational modifications, such as glycosylation and phosphorylation
> Lipoprotein profiling
> Peptide mapping
> Analysis of folding variants
> Analysis of oligosaccharides
> Monomer and aggregate determination
> Enzyme/isoform profiling

We offer a range of solutions that include analytical high resolution chromatography techniques for selectivity, speed and robustness.



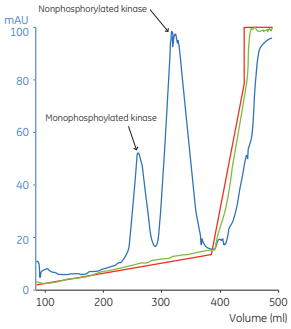
Separation of differentially phosphorylated kinase isoforms

Products featured: Mono S HR 10/10, Mono Q HR 5/5, ÄKTApurifier 100

One of the key factors for successful protein crystallography is homogenicity; small amounts of contaminants are far better tolerated than different forms of the same protein. Such variations may be caused by truncation, different isoforms, or post-translational modifications such as glycosylation, phosphorylation, etc.

Mono S™ and Mono Q ion exchange media are highly suitable for the separation of differentially phosphorylated protein kinase isoforms, since resolution is a key factor. Staurosporine was added as a stabilizing inhibitor to enhance yield.

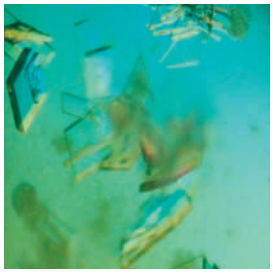
Separating phosphorylated and non-phosphorylated forms of ZAP-70 kinase



Column: Mono S HR 10/10*
Sample: 42 ml ZAP-70 kinase mixture
Start buffer: 20 mM $NaPO_4$, 5 mM DTT, 10 mM NaCl, 1 mM $MgCl_2$, pH 7.2
Elution buffer: Start buffer + 250 mM NaCl, pH 7.2
Flow rate: 2 ml/min
Gradient: 0-250 mM NaCl over 80 CV
System: ÄKTApurifier 100

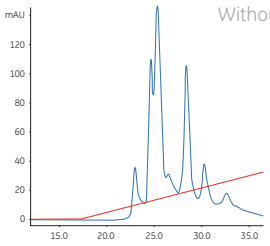
* Now replaced with Mono S 10/100 GL

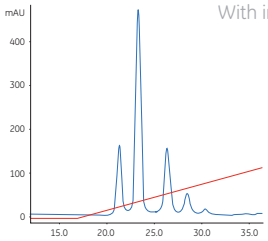
Two major peaks were eluted: a monophosphorylated kinase comprising 20% of the total ZAP catalytic domain, and a non-phosphorylated protein, eluting slightly later.



ZAP-70 crystals grown in the presence of staurosporine after purification.

Separation of different isoforms of a receptor tyrosine kinase in the presence and absence of staurosporine





Column: Mono Q HR 5/5**
Sample: 1.2 mg receptor tyrosine kinase in 5.4 ml in start buffer
Start buffer: 20 mM Tris-HCl, 5% v/v glycerol, 2 mM dithiothreitol, pH 8
Elution buffer: Start buffer + 1 M NaCl
Flow rate: 0.8 ml/min
Gradient: Linear 2.5% to 40% elution buffer in 37.5 CV
System: ÄKTApurifier

** Now replaced with Mono Q 5/50 GL

Acknowledgements: P. Ramage, B. Mathis, G. Fendrich and R. Benoit, Novartis Institutes for Biomedical Research, Basel, Switzerland.

Summary
For high-resolution separation of different phosphorylated forms of protein kinase, sample loading should be relatively low and shallow gradients should be used. Both yield and recovery can be increased by adding stabilizing inhibitors.

Analysis of glycoforms

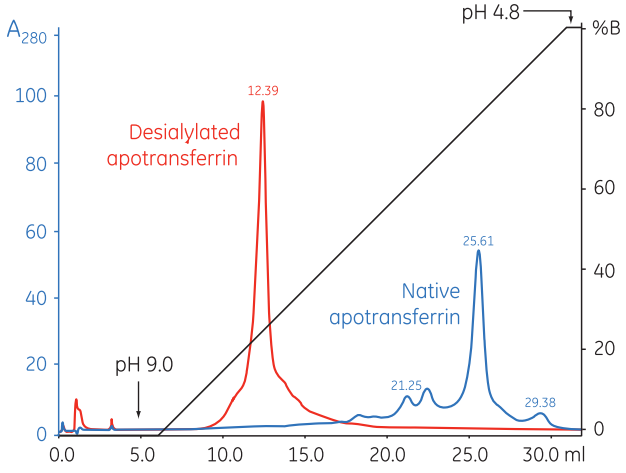
Products featured: Mono Q 5/50 GL, ÄKTAexplorer 10

Recent advances in glycoengineering, especially in yeast, will lead to increased demand for purification of recombinant glycoproteins. One of the major bottlenecks during the purification and analysis of glycoproteins is that the final product is heterogeneous in the glycan moiety.

Anion exchange chromatography is often used to measure the concentration of carbohydrate-deficient transferrin (desialylated or asialo transferrin) to diagnose the chronic abuse of alcohol. In a healthy individual, tetra-sialo transferrin is the main isoform.

N-glycan chains of transferrin differ in the degree of branching with di-, tri-, and tetra-antennary structures. Each antenna terminates with a negatively charged sialic acid. When transferrin is analyzed on an anion exchanger such as Mono Q, the number of peaks observed corresponds to different sialylated forms.

Column: Mono Q 5/50 GL
Flow rate: 1 ml/min
Start buffer: 20 mM piperazine, pH 9.0
Elution buffer: 20 mM piperazine, pH 4.8
Gradient: Linear, 0% to 100%, elution buffer in 25 CV
System: ÄKTAexplorer 10



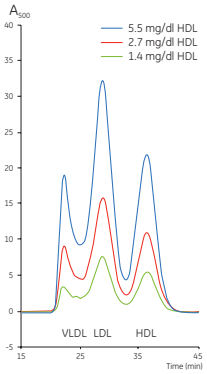
Separation of native apotransferrin (blue) and desialylated apotransferrin (red) by anion exchange chromatography using a pH gradient from 9.0 to 4.8.

Summary
High-resolution ion exchange chromatography with pH gradient elution can be used to separate different forms of a glycoprotein moiety.

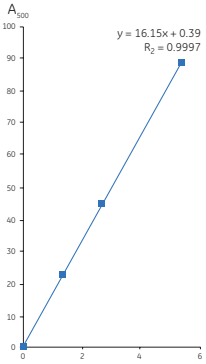
Lipoprotein profiling of individual serum samples

Products featured: Superose 6 PC 3.2/30, ÄKTAbasic 10

Lipoprotein profiling is an important analytical/clinical method for assessing the risks of contracting cardiovascular disease in individuals with risk factors such as obesity and metabolic diseases. Gel filtration is the method of choice for separation of lipoproteins such as VLDL (very low density lipoprotein), LDL (low-density lipoprotein), HDL (high-density lipoprotein), cholesterol, and triglyceride lipoproteins. In this example we present a highly sensitive method using ÄKTAbasic™ 10 and Superose™ 6 PC 3.2/30 columns to analyze lipoprotein profiles.



Different dilutions of human lipoproteins with internal HDL cholesterol standard (LabBo Immunosystems), injection volume: 10 µl.



Calibration curve for HDL, injection volume: 10 µl.

Column: Superose 6 PC 3.2/30 (2.4 ml)

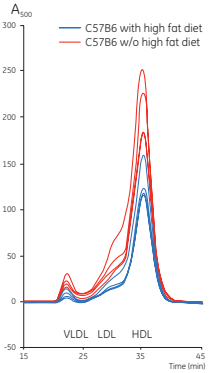
Flow rate: 50 µl/min

Sample: 5-10 µl of 1:1 or 1:2 diluted serum or plasma

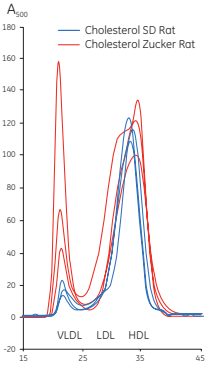
Buffer: 10 mM phosphate buffer, 138 mM NaCl, 2.7 mM KCl, 0.2 mM EDTA, pH 7.4

Elution volume: 2.5 ml

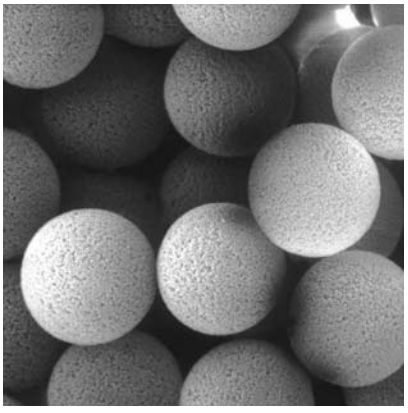
System: ÄKTAbasic 10



Individual lipoprotein profile of C57B6 mice fed normal and fat diet; injection volume: 10 µl of diluted serum in mobile phase 1:2 v/v.



Individual lipoprotein profiles of Sprague-Dawley and Zucker rats, injection volume: 10 µl of diluted serum in mobile phase 1:2 v/v.



Acknowledgement: C. Piveteau and J.M. Linget, CareX, Parc d'Innovation, Illkirch, France.

Summary

This sensitive analysis can measure as little as 1.4 mg/dl of HDL cholesterol, which represents an injected quantity of 140 ng of HDL, allowing the analysis of individual animals.

Separation of peptides: two folding variants of SOD

Products featured: SOURCE 5RPC ST 4.6/150, ÄKTAexplorer 10

Human extracellular superoxide dismutase (EC-SOD) exists in two folding variants denoted aEC-SOD and iEC-SOD, which are maintained by distinct disulfide bridge patterns. Tryptic digest of EC-SOD (S-carboxyamidomethylated) was purified by reversed phase chromatography and the distinct disulfide-linked peptides were identified by mass spectrometry.

Three cysteine-containing peptides of interest generated by tryptic digestion of EC-SOD

36- 59	DDDGTLHAACQVQPSATLDAAQPR (Cys45)	2480.65 Da
94-134	AIHVHQFGDLSQGCESTGPHYNPLAVPHPQHPGDFGNFAVR (Cys107)	4435.87 Da
187-202	LACCVVGVCGPGLWER (Cys189/190/195)	1660.02 Da

The masses of the disulfide-linked peptides

aEC-SOD (triple peptide): 2480 + 4435 + 1660 (S-carboxyamidomethylated , + 57 Da)	~ 8632 Da
iEC-SOD (double peptide): 4435 + 1660 (no S-carboxyamidomethylat ion) Da.	~ 6095 Da

Column: SOURCE™ 5RPC ST 4.6/150

Start material: Tryptic digest of EC-SOD purified from human aorta by affinity chromatography using Heparin Sepharose and ion exchange using a HiTrap Q HP 1ml column

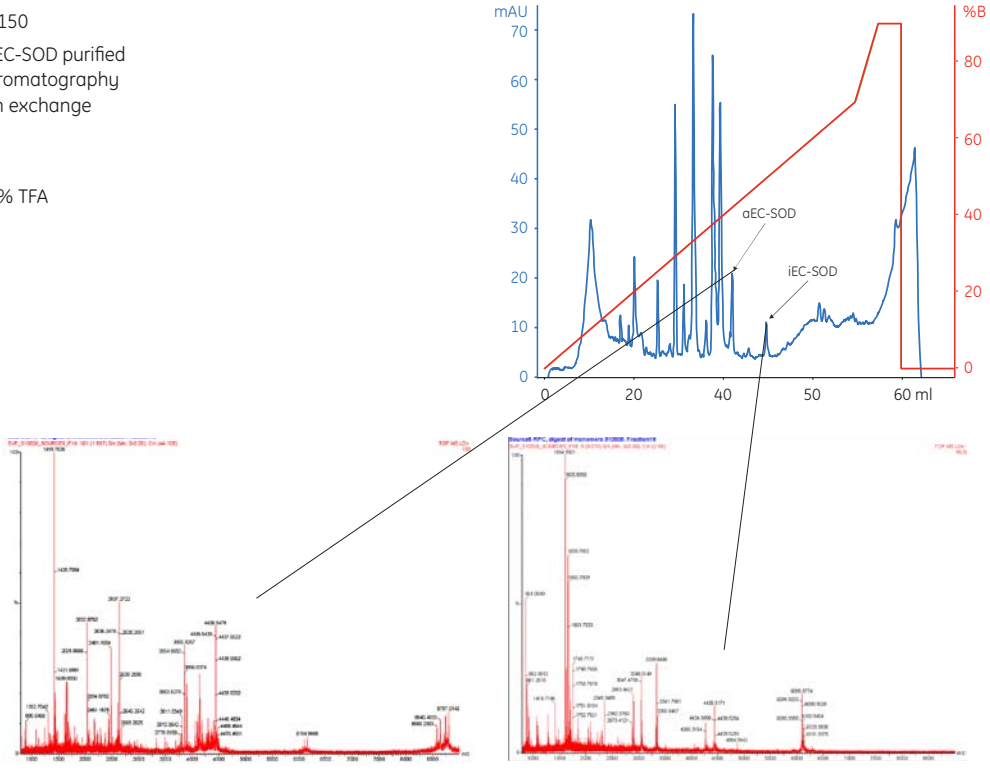
Solvent A: 0.1% TFA in water

Solvent B: 90% acetonitrile, 0.08% TFA

Gradient: 1% B/min

Flow rate: 200 µl/min

System: ÄKTAexplorer 10



Reference: Oury, T.D., *et al. Biochem J.* **317 (1)**: 51-57 (1996). **Acknowlegement:** S.V. Petersen and J.J. Enghild, Laboratory for Proteome Analysis and Protein Characterization, Dept. of Molecular Biology, University of Aarhus, Denmark.

Summary

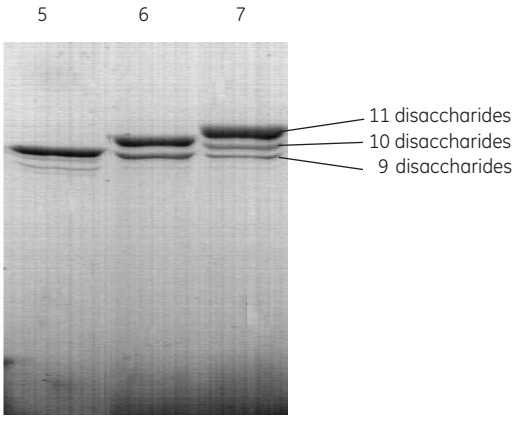
Separation of peptide peaks representing aEC-SOD and iEC-SOD was achieved using the SOURCE 5RPC ST 4.6/150 column. The disulfide-linked peptides, aEC-SOD and iEC-SOD, were identified by mass spectrometry.

Separation of oligosaccharides of hyaluronan

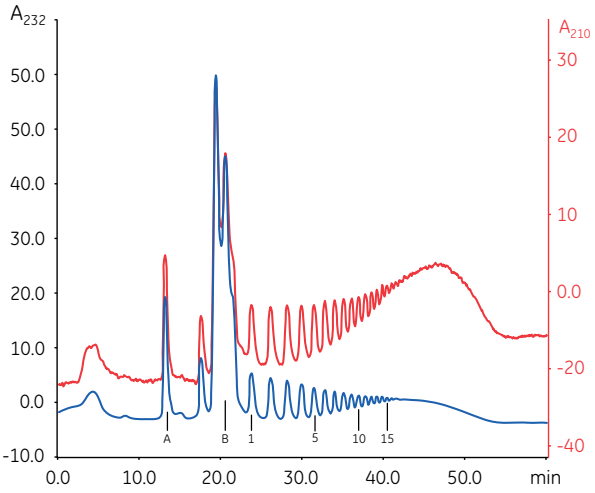
Products featured: Mono Q 4.6/100 PE, ÄKTApurifier 10

The separation of oligosaccharides of different lengths obtained from a digest of hyaluronan was investigated. Hyaluronan was digested with chondroitinase ABC (*Proteus vulgaris*) and applied to an anion exchanger, Mono Q. The hyaluronan oligosaccharide peaks were expected to separate according to increasing length and negative charge. To check the size range of oligosaccharides, eluted fractions were pooled broadly around the peaks. Several of the peaks were analyzed by fluorophore-assisted carbohydrate electrophoresis (FACE) and mass spectrometry.

Column: Mono Q 4.6/100 PE column
Start buffer: 20 mM Tris-HCl, pH 7.4
Elution buffer: 1 M NaCl in 20 mM Tris-HCl, pH 7.4
Flow rate: 1 ml/min
Gradient: 0% to 40 % B in 60 min
System: ÄKTApurifier 10



FACE analysis of peaks 5, 6, and 7 from Mono Q run. Each band corresponds to a hyaluronan of a defined length.



Analysis of partially digested hyaluronan by anion exchange chromatography using Mono Q. Peaks A and B were not detectable by FACE. Peaks 1 to 15 correspond to hyaluronan separated in size by one disaccharide.

Acknowledgement: C.S. Sonne-Schmidt, K.W. Sanggaard, and J.J. Enghild, Laboratory for Proteome Analysis, Dept. of Molecular Biology, University of Aarhus, Denmark.

Summary
Mono Q is an anion exchange medium that can be used to separate charged oligosaccharides of increasing lengths. A combination of mass spectrometry and FACE analysis indicates that purified fractions correspond to between 5 and 19 disaccharides corresponding to a molecular weight range between 1900 and 7220.

Guidelines for protein purification

Many chromatographers have come to rely on our extensive experience based on 50 years in the field. One of the key factors for success is keeping things simple. We hope that our guidelines for protein purification will provide a systematic framework for the development of purification strategies for virtually any biomolecule, and at any scale.

1. Define objectives

For required purity, activity, and quantity of final product to avoid overdeveloping or underdeveloping a method

2. Define properties of target protein and critical impurities

To simplify technique selection and optimization

3. Develop analytical assays

For fast detection of protein activity/recovery and critical contaminants

4. Minimize sample handling at every stage

To avoid lengthy procedures that risk losing activity or reducing recovery

5. Minimize use of additives

Additives can interfere with activity assays, and may need to be removed in an extra purification step

6. Remove damaging contaminants early

For example proteases

7. Use a different technique at each step

To take advantage of sample characteristics that can be used for separation (size, charge, hydrophobicity, ligand specificity)

8. Minimize number of steps

Extra steps reduce yield and increase time, so combine steps logically

Ordering information

Histidine-tagged proteins	Quantity	Code No.
His MultiTrap FF	4 × 96 well plates	28-4009-90
HisTrap FF crude	5 × 5 ml	17-5286-01
HisTrap FF crude	5 × 1 ml	11-0004-58
HisTrap FF	5 × 5 ml	17-5255-01
HiLoad 16/60 Superdex 200 pg	1	17-1069-01
His SpinTrap	50 × 100 µl	28-4013-53
His GraviTrap	10 × 1 ml	11-0033-99
HiLoad 26/60 Superdex 75 pg	1	17-1070-01
HiPrep, 26/10 Desalting	1	17-5087-01
Mono Q 5/50 GL *Replaces Mono Q HR 5/5*	1	17-5166-01
HisTrap HP	5 × 1 ml	17-5247-01

GST-tagged proteins	Quantity	Code No.
GST MultiTrap 4B	4 × 96 well plates	28-4055-00
GSTrap 4B	5 × 1 ml	28-4017-45
HiLoad 16/60 Superdex 200 pg	1	17-1069-01
GSTrap HP	5 × 1 ml	17-5281-01
HiLoad 16/60 Superdex 75 pg	1	17-1068-01
GSTrap FF	5 × 1 ml	17-5130-01
GSTrap FF	1 × 5 ml	17-5131-01
GSTPrep FF, 16/10	1	17-5234-01

Related literature and handbooks	Code No.
Recombinant Protein Purification Principles and Methods	18-1142-75
Antibody Purification	18-1037-46
Affinity Chromatography Handbook: Principles and Methods	18-1022-29
Gel Filtration: Principles and Methods	18-1022-18
Hydrophobic Interaction and Reversed Phase Chromatography: Principles and Methods	11-0012-69
Ion Exchange Chromatography and Chromatofocusing: Principles and Methods	11-0004-21
Protein Purification Handbook	18-1132-29
GST Gene Fusion System Handbook	18-1157-58

Antibodies	Quantity	Code No.
Ab SpinTrap	50 × 100 µl	28-4083-47
Protein A HP	5 × 5 ml	17-0403-03
HiTrap Protein G HP	5 × 1 ml	17-0404-01
HiLoad 16/60 Superdex 200 pg	1	17-1069-01
HiTrap MabSelect SuRe	5 × 1 ml	11-0034-93
HiPrep 26/10 Desalting	4	17-5087-02
HiTrap IgM Purification HP	5 × 1 ml	17-5110-01

Native and untagged proteins	Quantity	Code No.
HiTrap Capto Q	5 × 1 ml	11-0013-02
HiTrap Butyl FF	5 × 1 ml	17-1357-01
HiLoad 16/60 Superdex 200 pg	1	17-1069-01
HiTrap IMAC FF	5 × 1 ml	17-0921-02
HiTrap Desalting	5 × 5 ml	17-1408-01

Analytical separations	Quantity	Code No.
Mono S 10/100 GL	1	17-5169-01
Mono Q 5/50 GL	1	17-5166-01
Superose 6 PC 3.2/30	1	17-0673-01
SOURCE 5RPC ST 4.6/150	1	17-5116-01
Mono Q 4.6/100 PE	1	17-5179-01

Related literature and selection guides	Code No.
Ni Sepharose and IMAC Sepharose, selection guide	28-4070-92
Glutathione Sepharose, selection guide	28-9168-33
Affinity Chromatography Columns and Media, selection guide	18-1121-86
HiTrap Column Guide	18-1129-81
Gel Filtration Columns and Media, selection guide	18-1124-19
Ion Exchange Columns and Media, selection guide	18-1127-31
Prepacked Chromatography Columns with ÄKTAdesign Systems, selection guide	18-1173-49

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