GE Healthcare

Protein purification

Applications that meet your needs

You must deliver quality results, pure protein targets and publish findings—in time and on budget. While it is complex work, purification need never to be an obstacle for you. Better still, it offers you opportunities to gain real competitive advantages.

Here you'll find proven solutions to many of today's protein purification challenges. As well as an overview of the chromatography systems, services and quality assurances that we make available to support your science.

With nearly 50 years of experience and a wealth of knowledge in purifying proteins, the Protein Separations specialists at GE Healthcare are ready to help you gain real competitive advantages.

Contents

Histidine-tagged protein purification

About histidine-tagged proteins

Histidine-tag is the most used tag worldwide for recombinant proteins. The preparative purification of histidine-tagged recombinant proteins by Immobilized Metal Affinity Chromatography (IMAC) is both popular and highly effective. IMAC exploits the ability of the amino acid histidine to bind chelated transition metal ions, such as nickel (Ni^{2+}). Histidine-tag purification using IMAC can be performed under both native and denaturing conditions.

Purification challenges

It is important to get as high yield as possible of the active target protein. A high binding capacity saves you time and reduces consumption of purification media and buffers. Having your target protein in its active form may require the addition of detergents and other additives. As such, the purification method and media must be compatible.

In general, by increasing the concentration of imidazole in your binding and wash buffer you decrease unspecific binding. Conversely, by decreasing the concentration you get a higher capacity due to a stronger affinity interaction. The key is finding the optimal balance. Some target proteins require more purification steps to reach desired purity levels.

Solutions

Our solutions offer:

- Dramatically higher purification yields through up to four times higher binding capacity than previously available
- Minimized risk of inactive target proteins through excellent compatibility with a very wide range of reducing agents, detergents and other additives
- Reduced hands-on operations and greater flexibility delivered by convenient prepacked columns

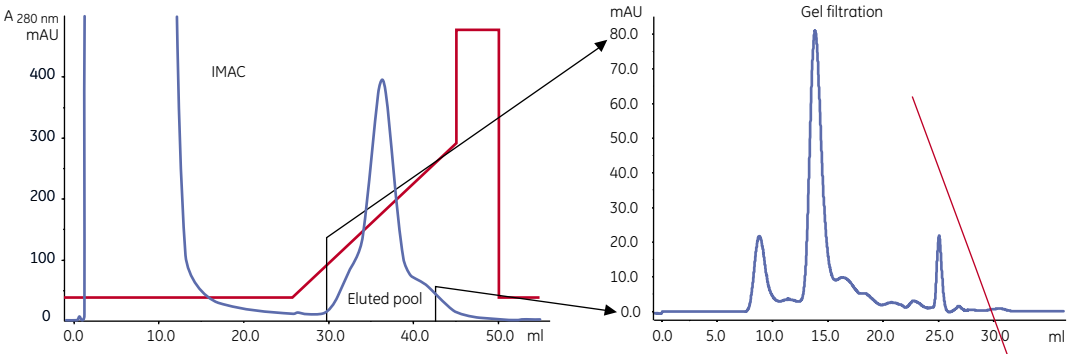
Two-step purification of a high molecular weight histidine-tagged protein

Fig. 1A: First purification step with IMAC

Sample: 10 ml *E. coli* extract with low-level expression of a histidine-tagged mannanase, Man 26A, from *Cellulomonas fimi* (M_r ~ 100 000)
Column: HisTrap HP 1 ml
Binding buffer: 20 mM sodium phosphate, 30 mM imidazole, 500 mM NaCl, pH 7.4
Elution buffer: 20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4
Gradient: 25 ml linear gradient 30-300 mM imidazole
Flow rate: 1ml/min
System: ÄKTAexplorer™ 100

Fig. 1B: Second step with gel filtration

Sample: 0.5 ml concentrated sample from IMAC step
Column: Superdex™ 200 10/300 GL
Buffer: PBS, pH 7.5
Flow rate: 0.5 ml/min
System: ÄKTAexplorer 100



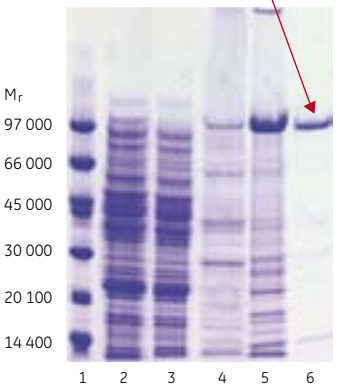


Fig. 1C: SDS-PAGE
Lane 1: LMW
Lane 2: *E. coli* extract
Lane 3: IMAC flow-through
Lane 4: Early IMAC fraction
Lane 5: IMAC eluted pool
Lane 6: Gel filtration pool

Conclusions

- The high molecular weight protein histidine-tagged mannanase Man 26A was purified in its enzymatically active form
- Excellent binding properties of Ni Sepharose™ High Performance (HP)
- 60 mg of purified protein in a single run
- A second purification step using gel filtration with Superdex 200 was added for high purity needs

About Ni Sepharose HP

Ni Sepharose HP delivers narrow peaks and high target protein concentration. It gives:

- High performance purification
- High target protein concentration
- Can be used with a syringe, pump, or system

Ni Sepharose HP is availabile as bulk media and in expertly prepacked HisTrap™ HP and HisPrep™ HP columns.

Scaling up a histidine-tagged protein purification procedure

Fig. 2A: Scale-up purificaiton of a histidine-tagged protein

Sample: Histidine-tagged Maltose binding protein in *E. coli* extract (samples loaded contained 8, 40 and 160 mg, respectively

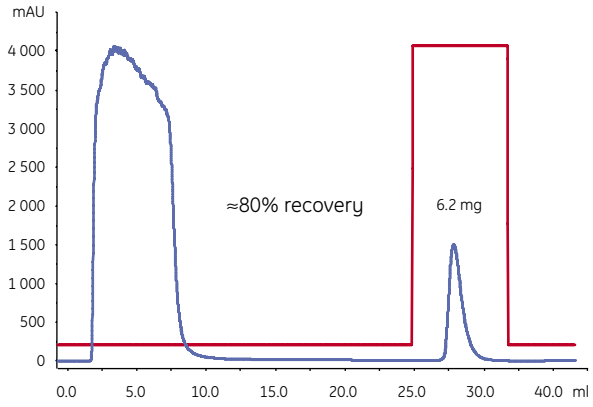
Columns: HisTrap FF 1 ml, HisTrap FF 5 ml, HisPrep FF 16/10 20 ml

Binding buffer: 20 mM sodium phosphate, 25 mM imidazole, 500 mM NaCl, pH 7.4

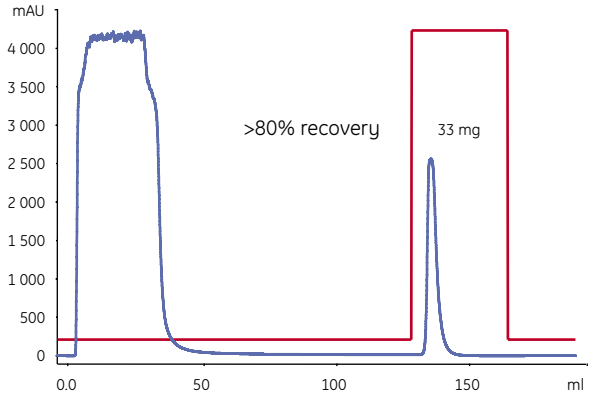
Elution buffer: 20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4

Flow rates: HisTrap FF 1 ml: 1 ml/min; HisTrap FF 5 ml: 5 ml/min; HisPrep FF 16/10: 5 ml/min

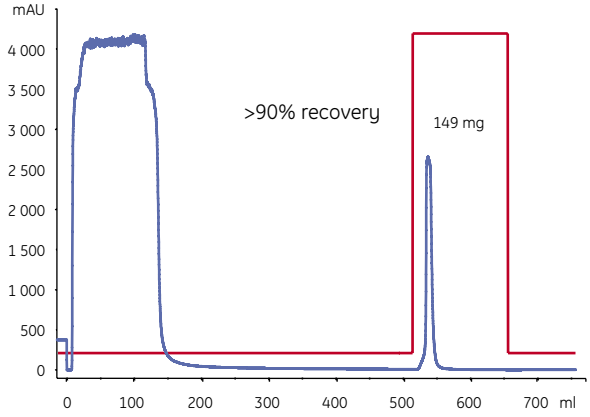
HisTrap FF 1 ml



HisTrap FF 5 ml



HisPrep FF 20 ml



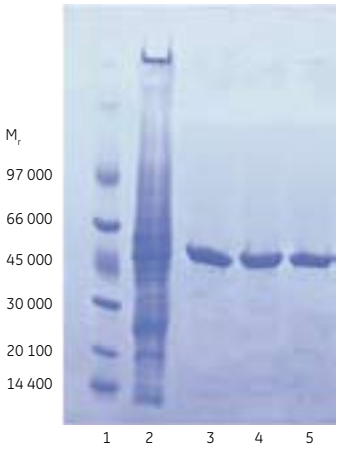


Fig. 2B: SDS-PAGE

Lane 1: LMW

Lane 2: Start material, *E. coli* extract

Lane 3: Eluted pool, HisTrap FF 1 ml

Lane 4: Eluted pool, HisTrap FF 5 ml

Lane 5: Eluted pool, HisPrep FF 16/10 (20 ml)

Conclusions

- Scale-up from HisTrap Fast Flow (FF) 1 ml via HisTrap FF 5 ml to HisPrep FF 16/10 (20 ml) is easy and efficient
- Scaling up column dimension while running at the same linear flow rate provides highly consistent results
- Pooled fractions analyzed by SDS-PAGE showed almost identical results in terms of purity and recovery
- Consistently high recovery and purity can be obtained in the different scales using the same flow rates

About Ni Sepharose 6 FF

Ni Sepharose 6 FF delivers fast flow rate purification and easy scale-up.

- Expression screening in multi-well plates
- Available expertly prepacked in convenient HisTrap and HisPrep prepacked columns as well as in bulk
- Manual purification, such as gravity flow and batch purification, and fast flow rate purification on systems

For high performance purification of proteins and narrow peaks with a chromatography system

Media, prepacked columns and kit	Quantity	Code no.
Ni Sepharose HP	25 ml	17-5268-01
Ni Sepharose HP	100 ml	17-5268-02
HisTrap HP	5 × 1 ml	17-5247-01
HisTrap HP	100 × 1 ml*	17-5247-05
HisTrap HP	1 × 5 ml	17-5248-01
HisTrap HP	5 × 5 ml	17-5248-02
HisTrap HP	100 × 5 ml*	17-5248-05
HisTrap HP kit	1	17-5249-01

Ni Sepharose FF

HisTrap FF

HisPrep FF

For high flow rate purification, scale-up and manual purification

Media and prepacked columns	Quantity	Code no.
Ni Sepharose 6 FF	25 ml	17-5318-01
Ni Sepharose 6 FF	100 ml	17-5318-02
Ni Sepharose 6 FF	500 ml	17-5318-03
HisTrap FF	5 × 1 ml	17-5319-01
HisTrap FF	100 × 1 ml*	17-5319-02
HisTrap FF	100 × 5 ml*	17-5255-02
HisPrep FF 16/10	1 × 20 ml	17-5256-01

Polishing gel filtration columns for even higher purity

Prepacked columns	Quantity	Code no.
Superdex 200 10/300 GL	1	17-5175-01
Superdex 75 10/300 GL	1	17-5174-01
Superdex Peptide 10/300 GL	1	17-5176-01
HiLoad™ 16/60 Superdex 200 pg	1	17-1069-01
HiLoad 16/60 Superdex 75 pg	1	17-1068-01
HiLoad 16/60 Superdex 30 pg	1	17-1139-01

Related literature

Poster	Code no.
Improved purification of histidine-tagged proteins with a new IMAC medium	11-0008-47

Data File	Code no.
Ni Sepharose 6 Fast Flow	11-0008-86
Ni Sepharose High Performance	18-1174-40

Selection Guide	Code no.
HiTrap™ columns	18-1129-81

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GST-tagged protein purification

About GST-tagged proteins

Glutathione S-transferase (GST) is a versatile means for expression, purification and detection of tagged proteins. GST-tagged proteins are purified by affinity chromatography utilizing immobilized glutathione, such as Glutathione Sepharose HP and FF.

Purification challenges

A high level of purity for required in most applications. High specificity between the ligand and tag is needed to deliver a highly pure protein. The target protein is bound by the specific affinity medium, impurities are washed away, and the protein target is eluted. Mild elution conditions are used when working with GST-tagged proteins to retain protein activity.

Solutions

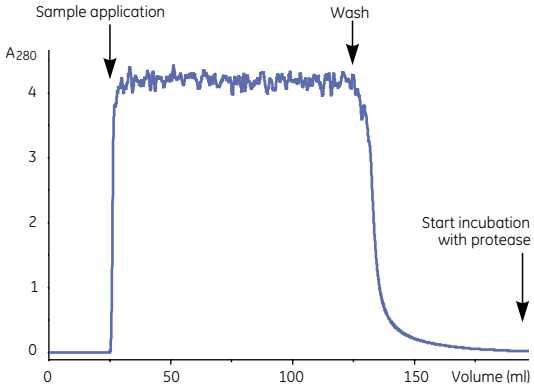
Our GST-tagged purification is based on Glutathione Sepharose. Glutathione Sepharose HP is for high performance and high resolution purification of GST-tagged proteins. Glutathione Sepharose FF is for high flow rate purification and easy scale-up. They offer:

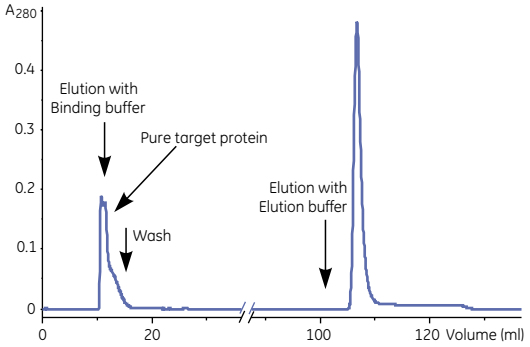
- Increased yield and purity using a one-step protocol for high selectivity
- Simple purification of GST-tagged proteins and other glutathione-S-transferase or glutathione-dependent proteins
- Elution under mild, non-denaturing conditions using reduced glutathione to preserve protein antigenicity and function
- Tag removal and purification at 4° C for improved stability
- The GST-tag can increase expression yield and solubility of your protein

One-step purification and on-column cleavage of a GST-tagged protein

Fig. 3A: Purification of a SH2 domain with concomitant removal of the GST tag

Sample:	100 ml clarified *E. coli* extract expressing SH2-domain GST-tagged protein (M_r 37 000)
Column:	GSTrap 5 ml
Binding buffer:	20 mM phosphate, 150 mM NaCl, pH 7.3
Elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Cleavage protease:	20 U/ml Thrombin Protease for 14 hours at room temperature
Flow rates:	10 ml/min (sample application and washing) and 2.5 ml/min (elution)
System:	ÄKTAexplorer 10





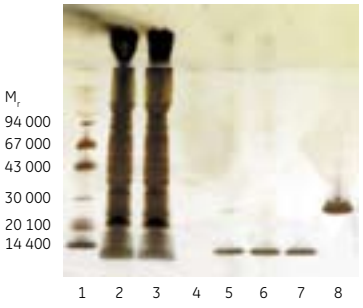
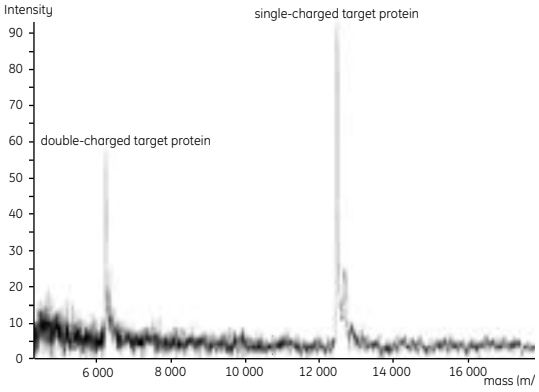


Fig. 3B: SDS-PAGE

- **Lane 1:** LMW
- **Lane 2:** Starting material, *E. coli* extract
- **Lane 3:** Flow-through fraction
- **Lane 4:** Last wash fraction
- **Lane 5:** SH2-domain without GST-tag eluted with binding buffer, first part of peak
- **Lane 6:** as lane 5, middle part of peak
- **Lane 7:** as lane 5, latter part of peak
- **Lane 8:** Cleaved off GST-tag by elution buffer

Fig. 3C: MALDI-TOF Mass Spectrometry (MS) analysis of the SH2 domain without GST-tag



Conclusions

- One-step purification of the SH2 domain on GSTrap FF 5 ml
- Highly pure target protein was obtained, as demonstrated by SDS-page and MALDI-ToF analysis
- On-column cleavage before elution
- Complete tag removal
- High flow rates (10 ml/min) allowed preparation to be completed four times faster

About Glutathione Sepharose 4 FF and GSTrap

- Glutathione Sepharose 4 FF medium provides excellent flow properties and is ideal for scaling up
- GSTrap™ 5 ml offers simple, one-step purification of GST fusion proteins, other glutathione S-transferases, and glutathione-dependent proteins
- Mild elution conditions preserve antigenicity and functionality
- Can be used with a syringe, pump, or system

Scaling up a GST-tagged protein purification procedure

Fig. 4A: GSTrap FF 1 ml

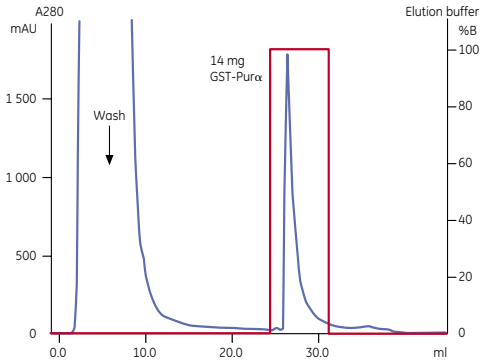


Fig. 4B: GSTrap FF 5 ml

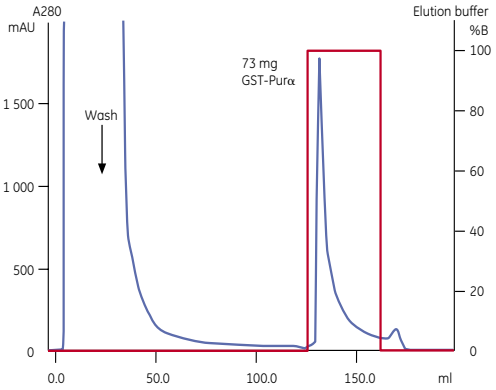
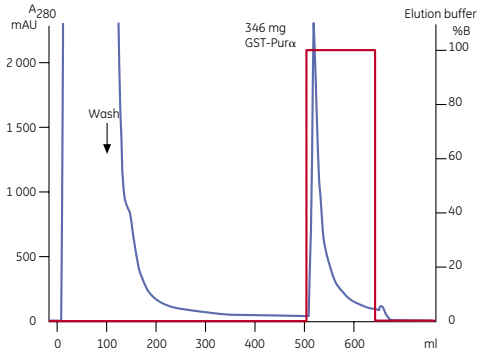


Fig. 4C: GSTPrep FF 16/10



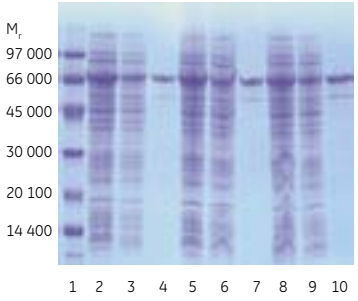


Fig. 4D: SDS-PAGE

Lane 1: LMW

Lane 2: Extract of *E. coli* expressing GST-purα, 1 g cell paste/ 5 ml

Lane 3: Flow-through from GSTrap FF 1 ml

Lane 4: GST-purα eluted from GSTrap FF 1 ml

Lane 5: As lane 2

Lane 6: Flow-through from GSTrap FF 5 ml

Lane 7: GST-purα eluted from GSTrap FF 5 ml

Lane 8: As lane 2

Lane 9: Flow-through from GSTPrep FF 16/10

Lane 10: GST-purα eluted from GSTPrep FF 16/10

Fig. 4A: GSTrap FF 1 ml

Sample:	5 ml *E. coli* extract expressing GST-Purα
Column:	GSTrap FF 1 ml
Binding buffer:	PBS, pH 7.4
Elution buffer:	50 mM Tris-HCl, pH 8.0 with 10 mM reduced glutathione
Flow rate:	Sample loading: 0.5 ml/min; washing and elution: 1 ml/min
System:	ÄKTAprime™

Fig. 4B: GSTrap FF 5 ml

Sample:	25 ml *E. coli* extract expressing GST-Purα
Column:	GSTrap FF 5 ml
Binding buffer:	PBS, pH 7.4
Elution buffer:	50 mM Tris-HCl, pH 8.0 with 10 mM reduced glutathione
Flow rate:	Sample loading: 2.5 ml/min; washing and elution: 5 ml/min
System:	ÄKTAprime

Fig. 4C: GSTPrep FF 16/10

Sample:	100 ml *E. coli* extract expressing GST-Purα
Column:	GSTPrep™ FF 16/10
Binding buffer:	PBS, pH 7.4
Elution buffer:	50 mM Tris-HCl, pH 8.0 with 10 mM reduced glutathione
Flow rate:	Sample loading: 5 ml/min; washing and elution: 10 ml/min
System:	ÄKTAprime

Conclusions

- Consistently high purity in the different scales
- Scale-up from GSTrap FF 1 ml via GSTrap FF 5 ml to GSTPrep™ 16/10 delivers reproducible purification results
- Two or more prepacked GSTrap FF 1 ml or 5 ml columns can easily be connected in series to increase binding capacity
- Note: it is important to keep the flow rate quite low during sample loading due to slow kinetics between the GST-tag and the ligand glutathione

About GSTPrep FF 16/10

- Prepacked 20 ml HiPrep™ columns with Glutathione Sepharose 4 FF for scale-up purification of recombinant GST-tagged proteins, other glutathione S-transferases and glutathione-dependent proteins
- Sepharose 4 FF matrix in combination with HiPrep column provide excellent flow properties
- Mild elution conditions preserve protein antigenicity and function

For high performance purification of GST-tagged proteins

Media and prepacked columns	Quantity	Code no.
Glutathione Sepharose HP	25 ml	17-5279-01
Glutathione Sepharose HP	100 ml	17-5279-02
GSTrap HP	5 × 1ml	17-5281-01
GSTrap HP	100 × 1 ml*	17-5281-05
GSTrap HP	1 × 5 ml	17-5282-01
GSTrap HP	5 × 5 ml	17-5282-02
GSTrap HP	100 × 5 ml*	17-5282-05

GSTrap HP

For high flow rate purification and scale-up of GST-tagged proteins

Media and prepacked columns	Quantity	Code no.
Glutathione Sepharose 4 FF	25 ml	17-5132-01
Glutathione Sepharose 4 FF	100 ml	17-5132-02
Glutathione Sepharose 4 FF	500 ml*	17-5132-03
GSTrap FF	2 × 1 ml	17-5130-02
GSTrap FF	5 × 1 ml	17-5130-01
GSTrap FF	1 × 5 ml	17-5131-01
GSTrap FF	5 × 5 ml	17-5131-02
GSTPrep FF 16/10	1 × 20 ml	17-5234-01

Glutathione Sepharose 4 FF and GSTrap FF

Automated chromatography system(s) cited

System	Quantity	Code no.
ÄKTAprime	1	18-1139-47
ÄKTAexplorer 10	1	18-1300-00

ÄKTAexplorer

Related literature

Poster	
Rapid purification of GST-fusion proteins from large sample volumes	18-1139-51

Data file	Code No.
Glutathione Sepharose 4 Fast Flow	18-1174-85
Glutathione Sepharose High Performance	18-1174-32
GST gene fusion system	18-1159-30

Selection Guide	Code No.
HiTrap columns	18-1129-81

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High throughput protein purification

About high throughput purification

The use of recombinant proteins has increased greatly over recent years. The incorporation of fusion tags simplifies the purification process and can also increase yield due to increased solubility of the expressed proteins. $(His)_6$ and GST, the most widely used tags for recombinant proteins, allow for protocol automation and rapid purification.

Purification challenges

To obtain the highest protein purity, purification protocols must include multiple steps. However, running many samples simultaneously through multi-step protocols is a tedious and time consuming process using traditional chromatography systems. Additionally, many downstream applications require the removal of the fusion tags, which increases the complexity of the protocol. Completely automating this process allows the user to concentrate on other important tasks.

Solutions

ÄKTAxpress™ is the first fully integrated automated platform for high throughput multi-step purification of tagged proteins. ÄKTAxpress is a modular platform that combines hardware, UNICORN™ software, columns and media. Depending on system configuration, ÄKTAxpress means you can:

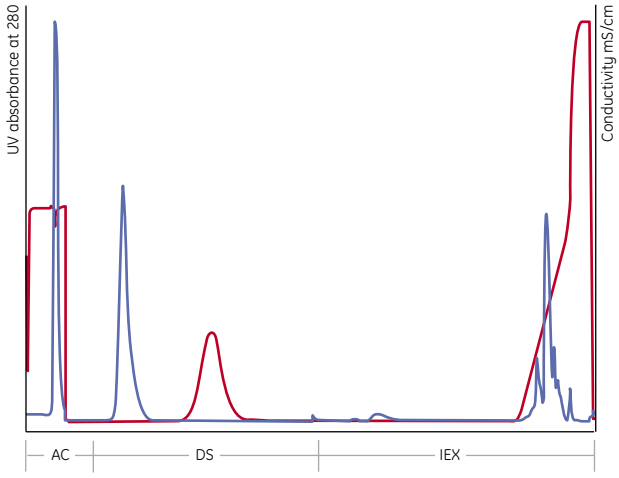
- Run up to 48 samples simultaneously
- Get up to 50 mg of up to >95% pure target proteins without manual intervention
- Use method wizards for creating and using pre-optimized protocols, encompassing on-column tag cleavage

Optimizing protocols for automated multi-step purification of histidine- and GST-tagged proteins

ÄKTAxpress purification protocols start with affinity chromatography, followed by combinations of desalting, ion-exchange and gel filtration. See Table 1 for a description of available multi-step protocols on ÄKTAxpress. An example from an automated three-step protein purification prototcol is presented in Fig. 5A. Flow-through fraction from sample loading and unselected peaks are saved in separate vessels (not shown). Intermediate peaks are stored in internal capillary loops and automatically injected onto the next column. Preparation and washing of columns are done during each run as a part of the purification protocol.

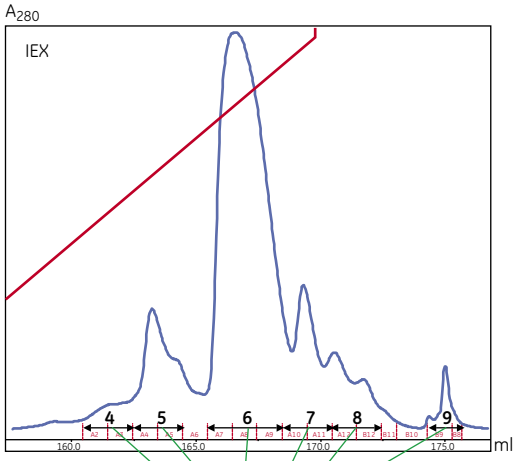
Fig. 5A: Automated multi-step purification of a histidine-tagged kinase

Sample:	Histidine-tagged kinase (Mr 42 200, pI 5.75) expressed in *E.Coli*
Columns	
AC:	HisTrap HP, 1ml
DS:	HiPrep™ 26/10 Desalting
IEX:	MonoQ™ 5/50 GL, 1ml
Buffers	
AC binding buffer:	50 mM Tris-HCl, 500 mM NaCl, 5 mM imidazole, 1 mM DTT, 10 % glycerol, pH 8
AC elution buffer:	50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, 1 mM DTT, 10 % glycerol, pH 8
DS and IEX binding buffer:	50 mM Tris-HCl, 25 mM NaCl, 1mM DTT, 10 % glycerol, pH 7.5
IEX elution buffer:	50 mM Tris-HCl, 1 M NaCl, 1mM DTT, 10 % glycerol, pH 7.5



AC: Affinity chromatography, **DS:** Desalting, **IEX:** Ion exchange chromatography

Fig. 5B: Final step



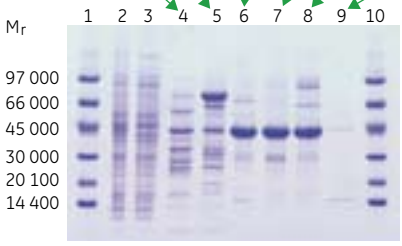


Fig. 5C: SDS-PAGE

Lane 1:	LMW
Lane 2:	Start sample
Lane 3:	Flow through
Lane 4–9:	See corresponding fractions in chromatogram
Lane 10:	LMW

Conclusions

- Automated multi-step purification of a histidine-tagged protein kinase
- Final step (Fig. 5B) shows different phosphoforms of the purified kinase and very high purity
- Method wizard for easy creation of purification protocols
- Effective automated peak detection and collection in intermediate steps

Table 1: Available multi-step protocols supported by ÄKTAxpress

Protocols	Effects of the additional chromatographic steps
AC–DS	Buffer exchange
AC–GF	Separation from undesired aggregates and contaminants
AC–DS–IEX	Separation from other isoforms (e.g. heterogeneously phosphorylated or glycosylated proteins)
AC–DS–IEX–DS	Separation from other isoforms on IEX and buffer exchange on DS
AC-DS-IEX-GF	Separation from other isoforms on IEX and removal of undesired aggregates and contaminants on GF

AC: Affinity chromatography
DS: Desalting
GF: Gel filtration
IEX: Ion exchange chromatography

Automated tag removal using ÄKTAxpress

Columns:

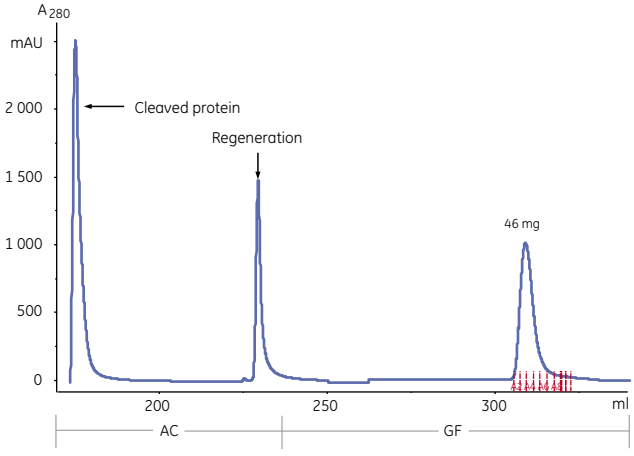
AC: HisTrap HP 5 ml [for $(His)_6$-tagged proteins]
AC: GSTrap HP 5 ml [for GST-tagged proteins]
DS: HiPrep 26/10 Desalting, 53 ml
IEX: RESOURCE™ Q, 6 ml
GF: HiLoad 16/60 Superdex 75 prep grade, 120 ml

Buffers:

AC (His) binding buffer:	50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.5
AC (His) cleavage buffer:	50 mM Tris-HCl, 500 mM NaCl, 50 mM imidazole, pH 7.5
AC (His) elution buffer:	50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.5
AC (GST) binding and cleavage buffer:	50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5
AC (GST) elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
DS and IEX binding buffer:	50 mM Tris-HCl, pH 8.0
IEX elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 8.0
GF buffer:	50 mM Tris-HCl, 150 mM NaCl, pH 7.5

Fig. 6B: Automated cleavage and purification using PreScission™ protease

Sample: GST-pura; GST-tagged
Two-step protocol: AC–GF



AC: Affinity chromatography, **GF:** Gel filtration

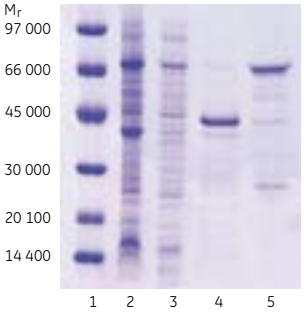
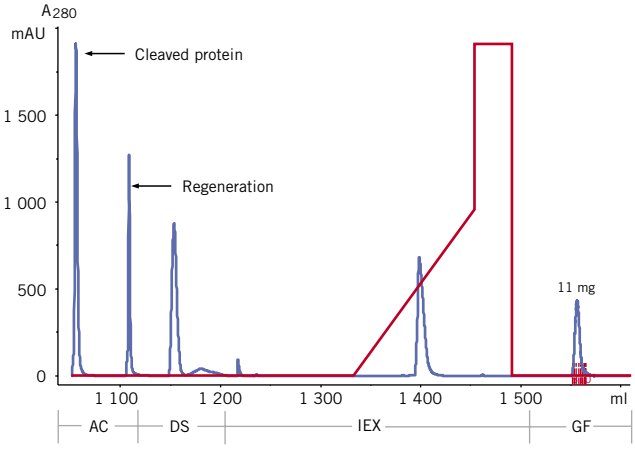


Fig. 6D: SDS-PAGE

Lane 1: LMW
Lane 2: Start sample
Lane 3: Flow through
Lane 4: Purified, cleaved GST-pura (M_r 35.2 x 10^3) after AC–GF
Lane 5: Reference: uncleaved GST-pura (M_r 61.6 x 10^3)

Fig. 6A: Automated cleavage and purification using a AcTEV Protease

Four-step protocol: 4-steps: AC–DS–IEX–GF
Sample: 0. 5 mg, M_r 61 600 (cleaved product M_r 35 200) APC1040; $(His)_6$-tagged



AC: Affinity chromatography, **DS:** Desalting, **IEX:** Ion exchange chromatography, **GF:** Gel filtration

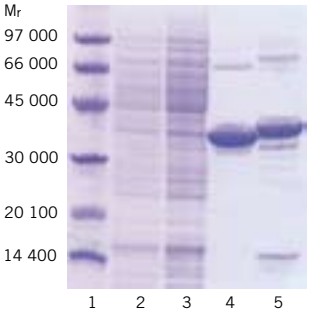


Fig. 6C: SDS-PAGE

Lane 1: LMW
Lane 2: Start sample
Lane 3: Flow through
Lane 4: Purified, cleaved APC1040 (M_r 36.4 x 10^3) after AC–DS–IEX–GF
Lane 5: Reference: uncleaved APC1040 (M_r 38.9 x 10^3)

Conclusions

- Fully-automated tag removal using PreScission and AcTEV Protease
- Yields of tens-of-milligrams without the fusion protein were obtained
- All processed proteins were of high purity

About ÄKTAxpress

- Two- to four-step protocols deliver the highest possible purity (up to >95%)
- Automatic tag removal is possible with all protocols
- High throughput: 16 samples overnight using a two-step purification, eight samples a day using a four-step purification protocol on a four-module system
- Automation eliminates time consuming manual tasks
- Up to four samples can be purified simultaneously per module
- Up to 12 modules can be controlled from one computer

High throughput systems for unattended multi-step operations

System	Quantity	Code no.
ÄKTAxpress, four module system with computer	1	18-6645-05
ÄKTAxpress TWIN, two module system with computer	1	11-0012-85

Affinity chromatography products for high throughput protein purification

Prepacked columns	Quantity	Code no.
HisTrap HP	5 × 1 ml	17-5247-01
HisTrap HP	100 × 1 ml*	17-5247-05
HisTrap HP	5 × 5 ml	17-5248-02
HisTrap HP	100 × 5 ml*	17-5248-05
GSTrap HP	5 × 1 ml	17-5281-01
GSTrap HP	100 × 1 ml*	17-5281-05
GSTrap HP	5 × 5 ml	17-5282-02
GSTrap HP	100 × 5 ml*	17-5282-05

Desalting products for high throughput protein purification

Prepacked columns	Quantity	Code no.
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29

Ion exchange products for high throughput protein purification

Prepacked columns	Quantity	Code no.
Mono Q 5/50 GL	1 × 1 ml	17-5166-01
Mono S™ 5/50 GL	1 × 1 ml	17-5168-01
RESOURCE Q	1 × 1 ml	17-1177-01
RESOURCE S	1 × 1 ml	17-1178-01
RESOURCE Q	1 × 6 ml	17-1179-01
RESOURCE S	1 × 6 ml	17-1180-01

Gel filtration products for high throughput protein purification

Prepacked columns	Quantity	Code no.
HiLoad 16/60 Superdex 75 pg	1 × 120 ml	17-1068-01
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01

Related literature

Data file	Code No.
ÄKTAxpress	18-1177-69 AB

Application note	Code No.
Automated on-column tag cleavage and multi-step purification of histidine-and GST-tagged proteins	11-0011-26
Optimizing protocols for automated multi-step purification of histidine and GST-tagged proteins using ÄKTAxpress	11-0011-25

* special pack size delivered to customer order

HisTrap and MonoBeads in ÄKTAxpress

ÄKTAxpress

UNICORN method wizard

Antibody purification

About antibody purification

There are growing numbers of general research, therapeutic and diagnostic applications for antibodies (MAbs) and their fragments. The high specificity of a MAbs is a significant advantage, particularly in therapeutic applications and immunoblotting. Polyclonal antibodies are commonly used as reagents in immunochemical techniques, using crude serum from different species as the source.

Purification challenges

MAbs can be produced both *in vivo* and *in vitro*. *In vivo* involves purification from ascites which contain many other proteins, including host proteins, lipids and cell debris. Separating out host proteins is usually challenging. That's because there is a high abundance of albumin, host proteins have both a similarity in charge characteristics and similar properties to immunoglobulin. Lipids in ascites may also clog the column if they are not first removed. MAbs produced in cell culture are diluted, so purification must concentrate the sample.

Solutions

Protein G and Protein A Sepharose media are designed for purification of monoclonal and polyclonal IgG from ascites, serum and cell culture supernatants. They offer:

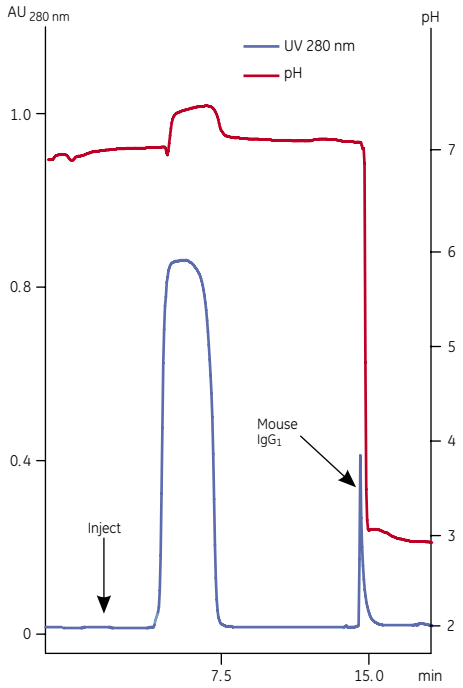
- Excellent purity (>95%) in one step
- High capacity for high yields
- High selectivity excludes most other proteins from binding
- Convenient prepacked columns and bulk packs

Despite gaining high purity from the affinity step, a polishing step using gel filtration is usually required to separate aggregates and/or dimers. Our Superdex medium is ideal for polishing and removing aggregates and/or dimers.

Purification of a mouse IgG_1 MAb from cell culture using Protein G

Fig. 7A: Capture step of Protein G purified mouse IgG_1 antibody

Sample: cell culture supernatant mouse IgG_1
Column: HiTrap Protein G HP 1 ml
Binding buffer: 20 mM sodium phosphate, pH 7.0
Elution buffer (B): 0.1 M glycine-HCl, pH 2.7
System: ÄKTAprime



Conclusions

- The automated two-step purification procedure using affinity and gel filtration chromatography is applicable for a wide range of general purification problems
- Protein G and Protein A Sepharose HP are designed for purification of monoclonal and polyclonal IgG from ascites, serum and cell culture supernatants
- Protein G and protein A, however, have different IgG binding specificities, dependent on the IgG origin (see table, pg. 17)

About Protein G Sepharose HP

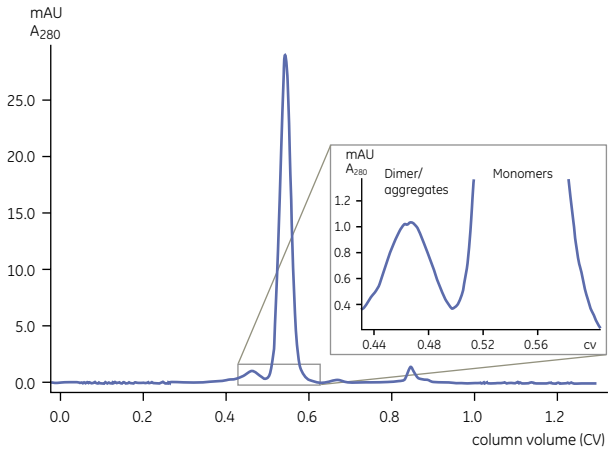
For convenient and rapid standard purification for monoclonal IgG antibodies:

- Binding of up to 25 mg human IgG/ml medium
- High capacity and fast binding kinetics
- Stability from pH 3-9 for use and 2-9 for cleaning
- Simple operations with a syringe, pump, or high performance chromatographic system, such as ÄKTAdesign™

Monomer/dimer separation of a MAb

Fig. 7B: Polishing of Protein G purified IgG_1 antibody using gel filtration

Column: Superdex 200 10/300 GL
Buffer: PBS, pH 7.2
Flow rate: 0.7 ml/min
System: ÄKTA$_{FPLC}$™



Conclusions

In most antibody preparations there is a possibility that IgG aggregates and dimers are present. Therefore, it is essential to include a gel filtration polishing step to get pure, homogenous MAbs. Superdex 200 gel filtration is an excellent medium for this purpose, as can be seen in Fig. 7B.

About Superdex 200

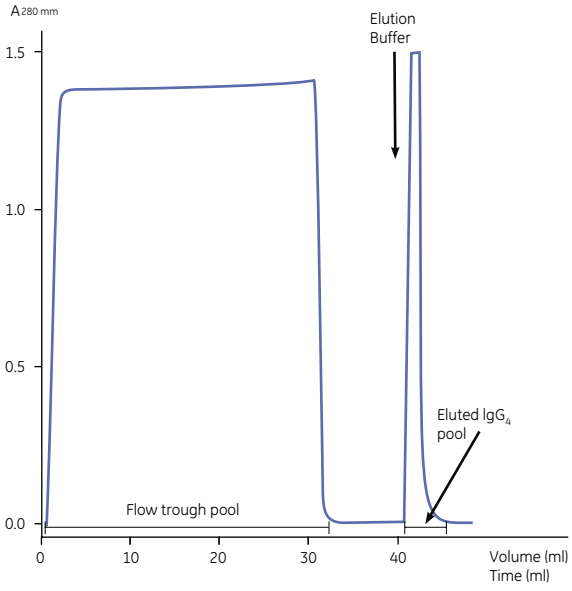
Superdex 200 medium is ideal for polishing and removing aggregates and dimers in MAb purification because:

- Separations in the range from M_r 10 000 up to 600 000 (globular proteins)
- Easy and predictable scale-up
- Excellent reproducibility and durability
- Available in expertly prepacked columns and as bulk media

Purification of humanized IgG_4 from cell culture using Protein A

Fig. 8A: Purification using HiTrap rProtein A FF 1 ml

Sample:	30 ml containing 12 mg of IgG_4
Column:	HiTrap rProtein A FF, 1 ml
Binding buffer:	200 mM sodium phosphate, pH 7.0
Elution buffer:	100 mM sodium citrate, pH 3.0
Flow:	1 ml/min (156 cm/h)
System:	FPLC™ System
Eluted amount of IgG_4:	11.2 mg
Yield:	93%
Purity:	>95% according to SDS-PAGE



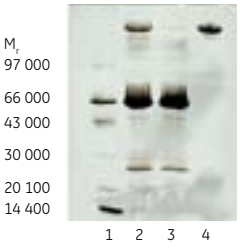


Fig. 8B: SDS-PAGE

Lane 1: LMW
Lane 2: Starting material
Lane 3: Flow-through pool
Lane 4: Eluted IgG_4 pool

Conclusions

- Humanised IgG_4 was purified directly from a myeloma cell culture, resulting in a highly purified MAb with 93% yield
- The purity from one-step purification was > 95%

About HiTrap Protein A Sepharose HP

- Prepacked with Protein A Sepharose HP for high capacity and selectivity purification and fractionation of IgG subclasses
- About 50 mg of human polyclonal IgG purified in one run
- Stable over pH 3-9 for use, and 2-10 for cleaning
- Simple operations with a syringe, pump, or high performance chromatographic system, such as ÄKTAdesign

Relative binding strengths: protein A and protein G

Here are the relative binding strengths of polyclonal immunoglobulins from various species to protein A and protein G, 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	no distinction	++++	+++
Rat	IgG_1	–	+
	IgG_{2a}	–	++++
	IgG_{2b}	–	++
	IgG_3	+	++
Sheep	IgG_3	+/-	++

* Purify using HiTrap IgM purification HP columns
† Purify using HiTrap IgY purification HP columns
- Weak or no binding

For applications requiring narrow peaks and high concentration

Media, prepacked columns and kit	Quantity	Code no.
HiTrap Protein A HP	5 × 1 ml	17-0402-01
HiTrap Protein A HP	2 × 1 ml	17-0402-03
HiTrap Protein A HP	1 × 5 ml	17-0403-01
HiTrap Protein G HP	5 × 1 ml	17-0404-01
HiTrap Protein G HP	2 × 1 ml	17-0404-03
HiTrap Protein G HP	1 × 5 ml	17-0405-01
MAbTrap™ Kit	1	17-1128-01

HiTrap Protein A

For applications requiring high yield and scale-up

Media and prepacked columns and media	Quantity	Code no.
rProtein A Sepharose 4 FF	5 ml	17-1279-01
rProtein A Sepharose 4 FF	25 ml	17-1279-02
rmp Protein A Sepharose FF	5 ml	17-5138-01
rmp Protein A Sepharose FF	25 ml	17-5138-02
Protein G Sepharose 4 FF	5 ml	17-0618-01
Protein G Sepharose 4 FF	25 ml	17-0618-02
HiTrap rProtein A FF	5 × 1 ml	17-5079-01
HiTrap rProtein A FF	2 × 1 ml	17-5079-02
HiTrap rProtein A FF	1 × 5 ml	17-5080-01

HiTrap Protein G

For removal of aggregates and polishing

Related products	Quantity	Code no.
HiLoad 16/60 Superdex 200 prep grade	1	17-1069-01
HiLoad 26/60 Superdex 200 prep grade	1	17-1071-01
Superdex 200 10/300 GL	1	17-5175-01
Superdex 200 prep grade	25 ml	17-1043-10
Superdex 200 prep grade	150 ml	17-1043-01

HiLoad Superdex

Automated chromatography system(s) cited

System	Quantity	Code no.
ÄKTA$_{FPLC}$	1	18-1118-67
ÄKTAprime	1	18-1139-47

Related literature

Data File	Code No.
HiTrap Protein A HP & HiTrap Protein G HP	18-1134-76
rProtein A Sepharose FF	18-1113-94
Protein G Sepharose FF	18-1012-91

Selection Guide	Code No.
HiTrap Columns	18-1129-81

Analytical separations

About analytical separations

After a protein is purified it is vital to find accurate techniques for the identification and characterisation of impurities and the target molecule. Common analyses tasks include:

- monomer/dimer determination
- enzyme activity
- purity and yield determination
- characterisation of a peptide map from a tryptic digest to identify post translational modifications

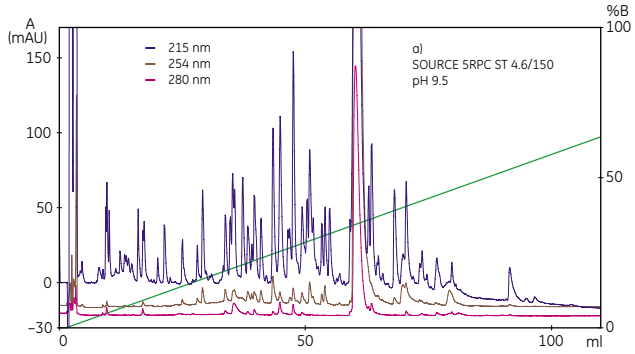
Solutions

- High resolution chromatography techniques offer high selectivity, speed, simplicity, and robustness
- An automated procedure encompassing sample injection and evaluation can be set up when many samples must be handled
- SOURCE™ RPC columns are ideal for applications requiring wide pH ranges

RPC separation for peptide mapping at high pH

Fig. 9A: Peptide mapping at pH 9.5 using SOURCE 5RPC ST 4.6/150

Sample: (RVP-BSA-mT), ca 750 pmol
Column: SOURCE 5RPC ST 4.6/150
Eluent A: 10 mM NH_4OH/HCOOH, pH 9.5
Eluent B: 60% acetonitrile in eluent A
Gradient: 0–67% B over 115 ml i.e. 46 column volume
Flow rate: 0.5 ml/min
System: ÄKTApurifier™ 10



Conclusions

- SOURCE 5RPC ST 4.6/150 proved very useful for alkaline purification of peptides, which are particularly difficult to dissolve in acidic solutions
- The polymeric matrix of the SOURCE medium makes it possible to perform RPC at high pH, offering high resolution and superior sensitivity for MS detection

About SOURCE 5RPC ST 4.6/150

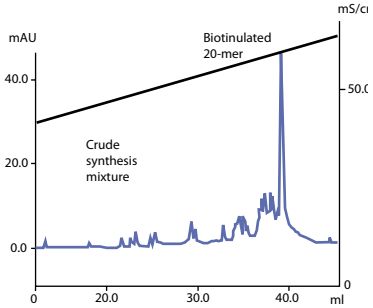
SOURCE 5RPC ST 4.6/150 stainless steel columns are designed for analytical reversed phase chromatography of peptides, protein fragments, and oligonucleotides. Thye offer:

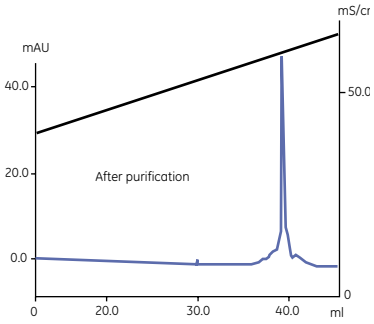
- Superior resolution in a very broad pH range (1-12)
- Outstanding performance at high pH
- Long lifetime due to high chemical and physical resistance
- High column-to-column reproducibility

Purity analysis of synthetic oligonucleotides

Fig. 9B: Purity check of a synthetic oligonucleotide

Sample: 5'-biotinylated synthetic oligonucleotide 20-mer
Column: Mini Q™ 4.6/50 PE
Start buffer: 10 mM NaOH
Eluent buffer: 10 mM NaOH, 2 M NaCl
Gradient: 0–67% B over 115 ml i.e. 46 column volume
Flow rate: 1.0 ml/min
System: ÄKTApurifier





Conclusions

- A powerful tool for purity check of synthetic oligonucleotides as Tricorn Mini Q™ is an anion exchange column and binds to the negative charged oligonucleotides

About Tricorn MiniBeads

- Tricorn™ high performance columns expertly prepacked with MiniBeads ion exchange chromatography media. They offer:
- Exceptional resolution with high reproducibility
- Extremely homogeneous 3 µm non-porous beads, yielding exceptional resolution, speed, reproducibility and durability
- Separation of proteins, peptides, oligonucleotides, carbohydrates, and other biomolecules according to charge using Mini Q and Mini S™
- Usage for both analytical and micropreparative applications

Analysis of glycated and non-glycated hemoglobin A_{1c}

Fig. 10A: Analysis of hemoglobin A_{1c}

Sample:	10 ml hemolyzed EDTA blood
Column:	Mono S™ 5/50 GL
Buffer A:	20 mM sodium malonate, 0.2 g/l sodium azide, pH 5.7
Buffer B:	buffer A + 0,3 M LiCl
Gradient:	20-50% B for 3 min; 50-75% B for 1.1 min, 75-100% B for 0.3 min, 100% B for 2.6 min, 20% B for 1 min
Flow rate:	2 ml/min

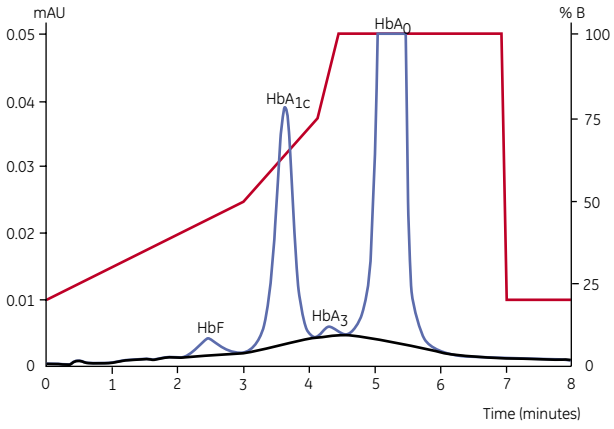
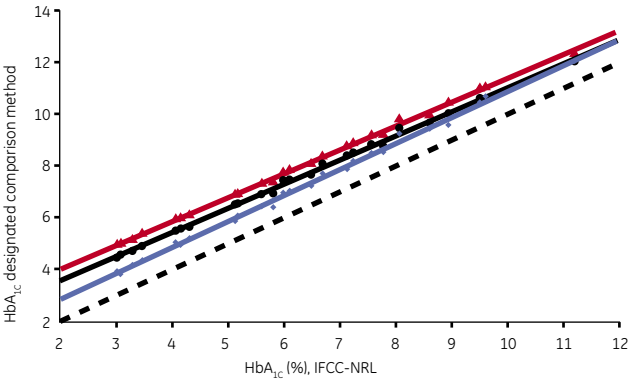


Fig 10B: Comparison of HbA_{1c} measured with nationally-designated methods and HbA_{1c} as defined by the IFCC method (4).



Conclusions

- Hemoglobin-A_{1c} (HbA_{1c}) is measured for evaluating the long-term control of diabetic patients
- Tricorn Mono S fully separates all different forms of glycosylated and non-glycosylated hemoglobin (Fig. 10A)
- The Mono S method has been used for two decades in Sweden to calibrate instruments that measure HbA_{1c}(1, 2). Fig. 7B shows that the Swedish-designated calibration method has highest resolution for the HbA_{1c} top and therefore lies closest to the standard set by the IFCC (3), in comparison against two other nationally-designated calibration methods.
- The Mono S method is a reliable and well-tested method for the measurement and calibration of HbA_{1c} instruments.

About Tricorn MonoBeads

Monodisperse porous beads expertly prepacked into Tricorn high performance columns for picogram to microgram-scale micropreparative and analytical separations of proteins peptides, protein fragments, and oligonucleotides. They offer:

- High resolution, reproducibility, and durability
- High purity levels for analysis and polishing
- Fast and simple operations using a high performance chromatography system, such as ÄKTAdesign

For RPC applications requiring wide pH ranges

Prepacked columns	Quantity	Code no.
SOURCE 5RPC ST 4.6/150	1	17-5116-01
SOURCE 15RPC ST 4.6/100	1	17-5068-01

SOURCE 5RPC

For analytical applications requiring high resolution and binding capacity

Prepacked Tricorn columns	Quantity	Code no.
Mono Q 5/50 GL	1	17-5166-01
Mono Q 10/100 GL	1	17-5167-01
Mono Q 4.6/100 PE	1	17-5179-01
Mono S 5/50 GL	1	17-5168-01
Mono S 10/100 GL	1	17-5169-01
Mono S 4.6/100 PE	1	17-5180-01

Tricorn MiniBeads

For analytical applications requiring extremely high resolution

Prepacked Tricorn columns	Quantity	Code no.
Mini Q 4.6/50 PE	1	17-5177-01
Mini S 4.6/50 PE	1	17-5178-01
Mini Q PC 3.2/3	1	17-0686-01
Mini S PC 3.2/3	1	17-0687-01

ÄKTApurifier

For analytical applications requiring high resolution and high recovery

Prepacked Tricorn columns	Quantity	Code no.
Superdex Peptide 10/300 GL	1	17-5176-01
Superdex 75 10/300 GL	1	17-5174-01
Superdex 200 10/300 GL	1	17-5175-01

For analytical applications requiring broad fraction range

Prepacked Tricorn columns	Quantity	Code no.
Superose™ 6 10/300 GL	1	17-5172-01
Superose 12 10/300 GL	1	17-5173-01

Automated chromatography system(s) cited

System	Quantity	Code no.
ÄKTApurifier 10	1	18-1400-00

Related literature

Data File	Code No.
SOURCE 5RPC ST 4.6/150 and ST 2.1/150	18-1132-36
Tricorn MonoBeads	18-1165-92
Tricorn MiniBeads	18-1165-93
Tricorn Superdex	18-1163-79
Tricorn Superose	18-1163-80

Application note	Code No.
Hemoglobin A_{1c} measurement with Mono S method	18-1167-92

Purification of non-tagged proteins

Purifying non-tagged proteins

Non-tagged protein purification can vary from simple one-step precipitation procedures to large scale, validated processes for biopharmaceutical manufacture. The need to purify a protein to sufficient purity and quantity levels in a simple, fast, reliable, and cost-effective manner is essential in all purification applications. A systematic approach can be used to develop a purification strategy.

Purification challenges

Successful protein purification requires following a multi-step approach to perform Capture, intermediate Purification and Polishing (CiPP). Specific objectives are assigned to each:

- 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

GE Healthcare offers a very wide range of lab-scale media and prepacked columns for purifying non-tagged proteins. These cover all major chromatographic techniques and steps. This allows 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
- Minimize sample handling between purification steps by combining the best techniques
- Increase yield while saving both time and money by using as few steps as possible

Rapid three-step purification of a labile, oxygen-sensitive enzyme

Fig. 11A: Capture: anion exchange chromatography

Sample: 40 ml vlarified *E. coli* extract of DAOCS, kept on ice

Column: HiPrep 16/10 Q XL

Start buffer (A): 50 mM Tris-HCl, 1 mM EDTA, 2 mM DTT, 0.2 M benzamidine-HCl, 0.2 mM PMSF, pH 7.5;

Elution buffer (B): A + 1.0 M NaCl

Gradient: 0% B in 5 column volumes, 30% B in 5 column volume, 100% B in 5 column volumes (step gradient)

Flow: 10 ml/min (300 cm/h)

System: ÄKTAFPLC

Fig. 11B: Intermediate purification: hydrophobic interaction chromatography

Sample: 40 ml DAOCS pool from HiPrep 16/10 Q XL, kept on ice

Column: SOURCE 15ISO, packed in HR 16/10 column

Start buffer (A): 1.6 M ammonium sulphate, 10% glycerol, 50 mM Tris-HCl, 1 mM EDTA, 2 mM DTT, 0.2 mM benzamidine-HCl, 0.2 mM PMSF, pH 7.5

Elution buffer (B): 50 mM Tris-HCl, 10% glycerol, 1 mM EDTA, 2 mM DTT, 0.2 mM benzamidine-HCl, 0.2 mM PMSF, pH 7.5

Gradient: 0–16% B in 4 column volume, 16–24% B in 8 column volume, 24–35% B in 4 column volume, 100% B in 4 column volume

Flow: 5 ml/min (150 cm/h)

System: ÄKTAFPLC

Fig. 11C: Polishing: gel filtration

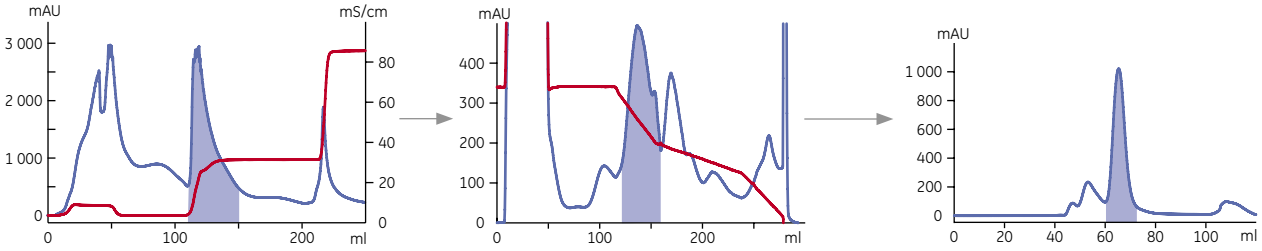
Sample: 3 ml DAOCS pool from SOURCE 15ISO, kept on ice

Column: HiLoad 16/60 Superdex 75 prep grade

Buffer: 100 mM Tris-HCl, 1 mM EDTA, 2 mM DTT, 0.2 mM benzamidine-HCl, 0.2 mM PMSF, pH 7.5

Flow: 1 ml/min (30 cm/h)

System: ÄKTAFPLC



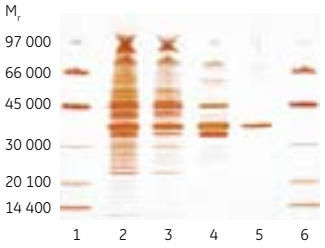


Fig. 11D: SDS-PAGE, silver stained

Lane 1: LMW

Lane 2: Start material *E. coli* extract

Lane 3: DAOCS pool from Q Sepharose XL

Lane 4: DAOCS pool from SOURCE 15ISO

Lane 5: DAOCS pool from Superdex 75 pg

Lane 6: LMW

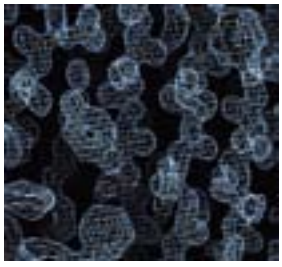


Fig. 11E: High resolution density map of purified DAOCS

Conclusions

- Rapid purification method with modern, preparative separation media and strategic purification design
- Isolation of 10 mg of an active, highly-labile enzyme to crystallization grade purity
- Timesavings of purification method: from three days to six hours
- Convenient optimization of separation conditions using pre-programmed method templates and scouting functions

About RESOURCE 15ISO

- Part of a range of columns prepacked with SOURCE media for fast and high-resolution separations
- Individual RESOURCE products feature ether (SOURCE 15ETH), isopropyl (SOURCE 15ISO) and phenyl (SOURCE 15PHE) hydrophobic ligands
- High performance at low back pressure, even with high sample loads of up to 25 mg of protein

About HiPrep 16/10 Q XL

- Prepacked with Sepharose Q XL or SP XL media
- 20 ml preparative anion- and cation-exchange columns provide high loading capacity and fast elution
- Optimized for reliable, reproducible separations

About HiLoad 16/60 Superdex 75 prep grade

- HiLoad 16/60 Superdex 75 prep grade is a prepacked XK column for preparative gel filtration separations
- Steep selectivity curves give excellent resolving power for peptides and proteins in M_r 3 000–70 000
- High mechanical strength with high hydrophilicity allow high flow rates and minimal nonspecific interactions

Two-step purification of a native protein at optimized pH

Fig. 12A: Capture and intermediate purification: HiTrap Q for anion exchange chromatography

Sample: 250 µl clarified estradiol (E2)-treated vitellogenin (Vtg) fish plasma sample
Column: HiTrap Q, 1 ml
Binding buffer: 0.1 M Tris–HCl, 1 mM PMSF, pH 7.0-8.5
Elution buffer: 0.1 M Tris-HCl, 1 mM PMSF, 0.5 M NACl, pH 7.0-8.5
Flow-rate: 5 ml /min
System: ÄKTApurifier 10

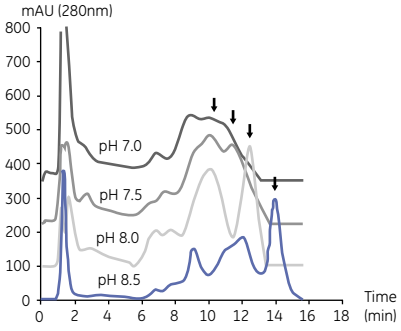
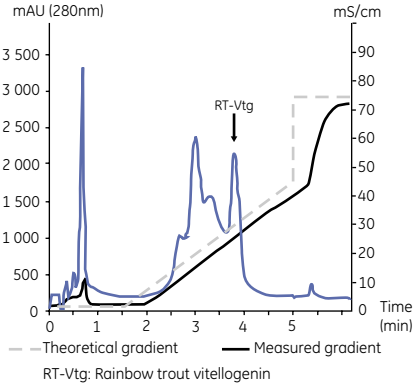


Fig. 12B: Elution profiles from pH optimized anion-exchange step

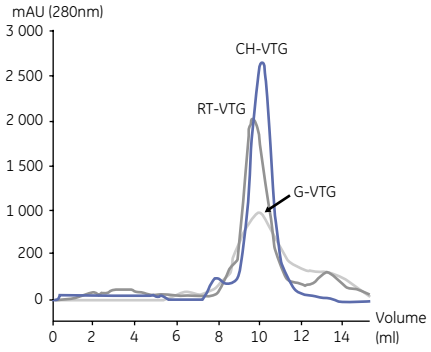
Sample: 10 ml clarified E2-treated fish plasma
Column: RESOURCE Q
Start buffer: 0.1 M Tris-HCl, pH 8.5
Elution buffer: 0.1 M Tris–HCl, 0.5 M NaCl, pH 8.5
Flow rate: 4 ml /min
System: ÄKTApurifier 10



RT-Vtg: Rainbow trout vitellogenin

Fig. 12C: Polishing using Superdex 200

Sample: Vtg fractions from anion-exchange pooled sample from RESOURCE Q of three E2-treated teleost fish
Elution buffer: 0.05 M carbonate–bi-carbonate, pH 9.6
Flow rate: 0.2 ml/min
System: ÄKTApurifier 10



RT-Vtg: Rainbow trout vitellogenin
CH-Vtg: Chub vitellogenin
G-Vtg: Gudgeon vitellogenin

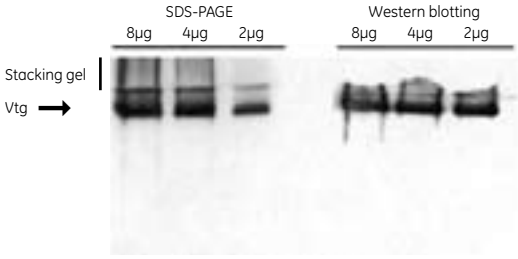


Fig. 12D: Native PAGE of purified rainbow trout vitellogenin and corresponding Western blotting after the GF-step

Electrophoresis was performed with a 4% stacking gel and 7.5% (w/v) resolving gel of acrylamide and silver stained. Immunoblotting was performed with BN-5 anti-salmon Vtg antibody; complexes were immunodetected by enhanced chemiluminescent method after transfer of proteins to a nitrocellulose paper.

Conclusions

- Vitellogenin (Vtg) was purified from three estradiol-treated teleost fish: rainbow trout, gudgeon and chub (5)
- Anion-exchange chromatography using HiTrap Q was used to optimize pH for capture and intermediate purification; high purity protein-binding capacity in pH 8.5 provided best resolution
- After pH optimization, RESOURCE Q was used for capture and intermediate purification during the routine purification procedure
- Superdex 200 was used in the final polishing step to further separate the degradation product from the native form of the Vtg and to store the final product in the desired buffer
- Combination of RESOURCE Q and Superdex improved reproducibility and decreased processing time

About RESOURCE Q and S columns

RESOURCE Q and S are prepacked columns with SOURCE 15 Q or 15 S for fast and simple ion exchange separations. They offer:

- High performance separations over a wide range of operating pressures
- Monodisperse 15 µm beads deliver excellent flow characteristics
- Minimal non-specific adsorption and high recovery of purified sample
- Relatively low back pressure at high flow rates, delivering high resolution separations even when using a low-pressure pump

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Products for purifying non-tagged proteins

GE Healthcare offers a very wide range of lab-scale prepacked columns and media for purifying non-tagged proteins. These cover all major chromatographic techniques and steps. Logical combinations of chromatographic steps is shown in Fig. 13A. A guide to the suitability of each technique for the stages of the Capture, intermediate Purification and Polishing (CiPP) strategy is shown in Table 13B.

If nothing is known about the target proteins, an effective general approach is: ion exchange (capture), hydrophobic interaction chromatography (intermediate purification) and gel filtration (polishing). For general information, please consult our Protein Purification Handbook or your local GE Healthcare representative. To identify the products that best suit your needs, please consult our selection guides, available at: **www.chromatography.amershambiosciences.com**

Fig. 13A: guide for combining chromatographic steps

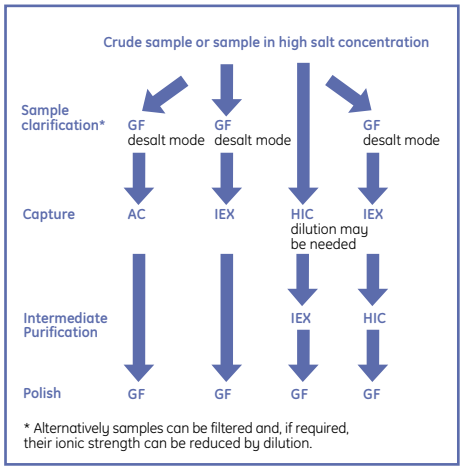


Table 13B: suitability of technique for the stages of the CiPP strategy

Technique	Main features	Capture	Intermediate	Polish	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	in organic solvent, risk loss of biological activity concentrated

AC: Affinity chromatography, **DS:** Desalting, **IEX:** Ion exchange chromatography, **GF:** Gel filtration, **HIC:** Hydrophobic Interaction chromatography, **RPC:** Reversed phase chromatography

Automated chromatography system(s) cited

System	Quantity	Code no.
ÄKTApurifier 10	1	18-1400-00
ÄKTA$_{FPLC}$	1	18-1900-26

Related literature

Selection guides and product profiles	Code no.
Ion exchange columns and media	18-1127-31
Gel filtration columns and media	18-1124-19
Affinity Chromatography columns and media	18-1121-86
Fast desalting and buffer exchange of proteins and peptides	18-1128-62
HiTrap column guide	18-1129-81
Prepacked chromatography columns with ÄKTAdesign systems	18-1173-49

Refolding proteins from inclusion bodies

About refolding recombinant proteins

For the expression of recombinant proteins, *E. coli* represents the most commonly used approach. Proteins are expressed either in cytoplasm or secreted. Over-expression of proteins in some cases leads to accumulation of the proteins in insoluble polypeptide aggregates, called inclusion bodies.

Purification challenges

To transform the proteins insoluble in within the inclusion bodies into a useful soluble, bioactive protein.

Solutions

Chromatographic on-column refolding offers:

- Automated procedures
- Reliable, simple scale-up
- Refolding and purification in a single step, simultaneously
- High compatibility and chemical stability with different additives, reducing agents, denaturing agents and detergents
- Convenient optimization of separation conditions using pre-programmed method templates and scouting functions

One-step on-column refolding and purification of a Histidine-tagged protein from *E. coli* inclusion bodies

Fig. 14A: On-column refolding and purification

Sample:	Histidine-tagged, solubilized single chain Fv antibody fragment Fab 57P, 10 ml (conc. 0.67 mg/ml) *E. coli* inclusion bodies
Column:	HisTrap HP 1 ml
Solubilizing buffer:	20 mM Tris-HCl, 6 M Gua-HCl, 1 mM DTE, 1mM Na_2-EDTA, 0.1 mM Pefabloc™, pH 7.5
Binding denatured buffer:	20 mM Tris-HCl, 5 mM imidazole, 0.5 M NaCl, 8 M urea, 1 mM DTE, 0.1 mM Pefabloc, pH 7.5
Refolding buffer:	20 mM Tris-HCl, 5 mM imidazole, 0.5 M NaCl, 0.5 M arginine-HCl, 1 mM reduced glutathione (GSH), 1 mM oxidised glutathione (GSSG), pH 7.5
Native binding buffer:	20 mM Tris-HCl, 10 mM imidazole, 0.5 M NaCl, pH 7.5
Native elution buffer:	20 mM Tris-HCl, 500 mM imidazole, 0.5 M NaCl, pH 7.5
System:	ÄKTAexplorer 10

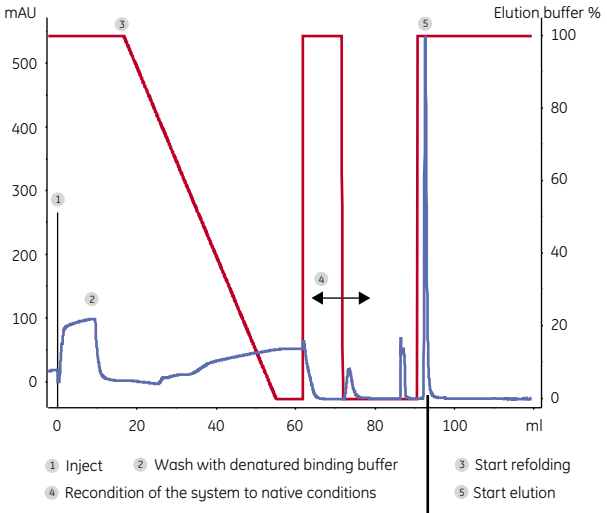


Fig. 14C: Pooled fraction from purification of refolded scFv 57P

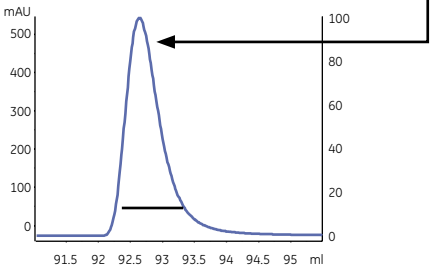
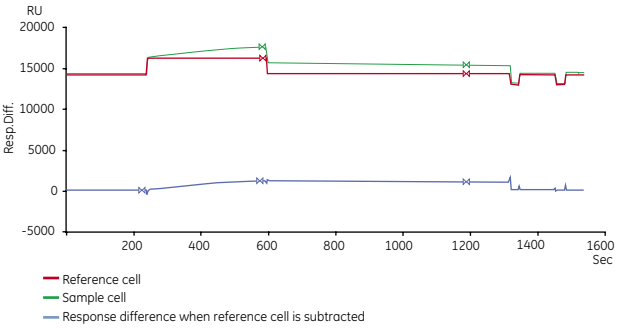


Fig. 14B: Sensogram of the interaction between immobilized peptide and refolded protein

System:	Biacore™ system 2000
Sensor surface:	Immobilized peptide C16V"37" (tobacco mosaic virus) with affinity to scFv57P
Blank surface:	Immobilized with peptide with no affinity to scFv57P
Yield:	14% active protein from pooled fraction



Conclusions

- Refolding yield was 14% from the purified refolded peak
- Refolding parameters of a histidine-tagged protein could be optimized simply and the final method automated for both refolding and purification with HisTrap HP

About HisTrap HP

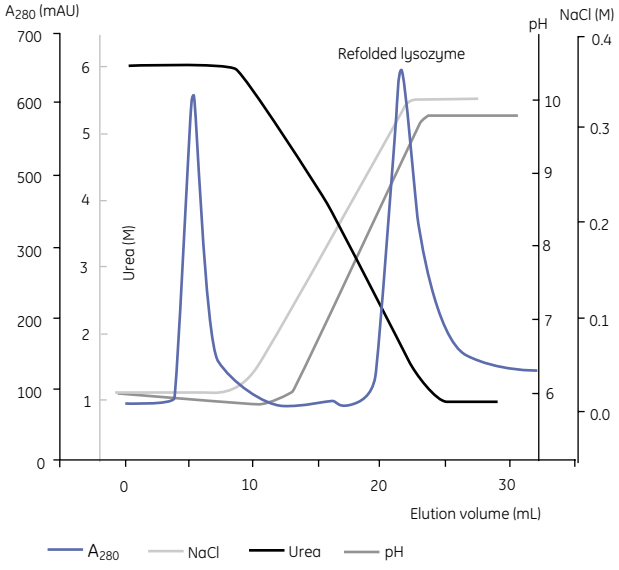
Ni Sepharose HP prepacked in convenient HisTrap columns for purification of histidine-tagged proteins. It offers:

- High binding capacity, at least 40 mg/ml medium
- Compatible with different additives, reducing agents, denaturing agents, and detergents
- Negligible Ni leakage.
- Simple operation with a syringe, pump, or high performance chromatography system, such as ÄKTAdesign

One-step on-column refolding and purification using a dual gradient

Fig. 15: Example of a dual-gradient ion-exchange refolding procedure.

Sample:	8 mg lysozyme dissolved in denaturing buffer
Column:	HiTrap SP HP 5 ml
Denaturing buffer:	50 mM Tris-HCl, 6 M urea, 3 mM GSH and 3 mM GSSG (redox ratio 1:1), pH 6.2
Refolding buffer:	100 mM Tris-HCl, 1 ml M urea, 0.3 M NaCl, 3 mM GSH and 3 mM GSSG (redox ratio 1:1), pH 10.0
Refolding dual gradient:	linear from denaturing- to refolding conditions in 5 ml gradient volume. pH changed from 6.2 to 10 in the refolding gradient at the same time
Flow rate:	0.4 ml/min



Conclusion

- Combining gradient with descending urea concentration gradient and ascending pH gradient gave higher recovery and activity of lysozyme using HiTrap SP Sepharose HP (8)
- Recovery of activity and mass of refolded protein were higher than in processes without gradient, or with only one gradient, for the refolding of denatured lysozyme (not shown)

About HiTrap SP Sepharose HP

SP Sepharose HP ion exchange media expertly prepacked in HiTrap columns for high resolution purification. It offers:

- Small particle size (34 µm) of the medium allows fast adsorption and desorption even at high sample loadings and flow rates
- High loading capacity over broad pH range
- Convenient format for fast, simple separations either alone or connected in series

Representative chromatographic refolding processes

There is no single refolding technique or method that satisfies all protein refolding requirements. Chemical conditions also vary from protein to protein. Several experiments are required to identify the optimal refolding process. Chromatographic refolding processes (see table below) have demonstrated their advantages for different proteins, denatured native proteins or polypeptides expressed as inclusion bodies (8)

Refolding mode	Chromatography mode	Elution mode	Protein	Recovery (%)	Reference
Solvent-exchange by GF	Normal gel filtration	Normal GF	RETS-1 isoform PDGF	75 75	[6] [7]
	Gradient gel filtration	No gradient Urea gradient Urea and pH gradients	ScFv	14.5 17.3 25	[8]
Solvent exchange during reversible adsorption	IEX	Three-buffer system Dual-gradient system	Some inclusion bodies Human lysozyme SOD	ND 50 40	[9-11] [12] [13]
	IMAC HIC RPC	Three-buffer system Normal HIC Normal RPC	Histidine-TNF Human interferon-α Human interleukin-2	90 ND ND	[14] [15] [16]
Use of an immobilized folding catalyst	GroEL, GroES	Mixture of denatured protein and medium	Lysozyme	85	[17, 18]
	Liposomes PEG	Normal chromatographic elution Normal chromatographic elution	Lysozyme Lysozyme	90 90	[19, 20] [21]

ND: not determined; **IEX:** Ion Exchange chromatography; **GF:** Gel Filtration; **IMAC:** Immobilized Metal Affinity Chromatography
HIC: Hydrophobic Interaction Chromatography; **RPC:** Reversed Phase Chromatography; **PEG:** Polyethylene glycol

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On-column refolding products

	Quantity	Code no.
HisTrap HP	5 × 1ml	17-5247-01
HisTrap HP	100 × 1 ml*	17-5247-05
HisTrap HP	1 × 5 ml	17-5248-01
HisTrap HP	5 × 5 ml	17-5248-02
HisTrap HP	100 × 5 ml*	17-5248-05
HiTrap SP HP	5 × 1ml	17-1151-01
HiTrap SP HP	5 × 5ml	17-1152-01
HiTrap Q HP	5 × 1ml	17-1153-01
HiTrap Q HP	5 × 5 ml	17-1154-01
HiTrap Phenyl HP	5 × 1 ml	17-1351-01
HiTrap Phenyl HP	5 × 5 ml	17-5195-01
RESOURCE ETH	1 × 1 ml	17-1184-01
RESOURCE ISO	1 × 1 ml	17-1185-01
RESOURCE PHE	1 × 1 ml	17-1186-01
RESOURCE RPC	1 ml	17-1182-01
HisTrap HP kit	1	17-5249-01

HiTrap SP Sepharose HP

HisTrap HP kit

ÄKTAexplorer

On-column refolding gel filtration products

Prepact columns	Quantity	Code no.
Superdex 75 HR 10/30	1	17-1047-01
Superdex 200 HR 10/30	1	17-1088-01
HiLoad 16/60 Superdex 30 prep grade	1	17-1139-01
HiLoad 26/60 Superdex 30 prep grade	1	17-1140-01
HiLoad 16/60 Superdex 75 prep grade	1	17-1068-01
HiLoad 26/60 Superdex 75 prep grade	1	17-1170-01
HiLoad 16/60 Superdex 200 prep grade	1	17-1069-01
HiLoad 26/60 Superdex 200 prep grade	1	17-1171-01

Automated chromatography system(s) cited

System	Quantity	Code no.
ÄKTAexplorer 10	1	18-1300-00

* special pack size delivered to customer order

Sample preparation

About sample preparation

Sample preparation encompasses a very wide variety of applications. For proteome analysis of human serum, many researchers are studying low abundance proteins. One of their primary goals is to find useful biomarkers for different diseases and conditions. Mass spectrometry (MS) analysis is a very important tool for protein analysis. Certain molecules degrade proteins in cell culture supernatants, bacterial lysate or serum.

Challenges

High abundance proteins, such as albumin and immunoglobulins, make it difficult to detect low abundance proteins. Proteases included in human plasma can damage the sample if not removed. MS analysis can not be performed when certain buffer salts are present in the solution.

Solutions

- Removal of high abundance proteins can be performed with HiTrap Blue HP columns
- HiTrap Benzamidine FF (high sub.) for removal of trypsin-like serum proteases from human plasma
- Desalting using Sephadex™ is very useful for removing buffer salts prior to purification steps and MS analysis
- Membrane purification utilizing cross-flow filtration for buffer exchange is ideal for larger volumes

Removal of high abundance proteins from human serum

Fig. 16A: HiTrap Desalting

Sample: 1 ml ISTH/SSC Human Plasma, Secondary Coagulation Standard
Column: HiTrap Desalting 5 ml
Buffer: 20 mM Tris-HCl, 100 mM NaCl, pH 7.4
Flow rate: 5 ml/min
System: ÄKTAFPLC

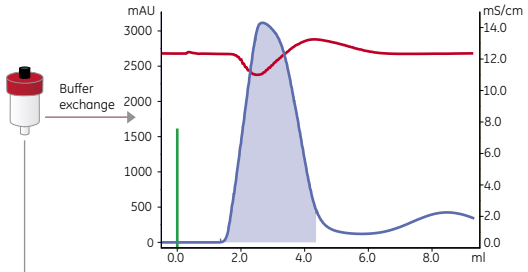
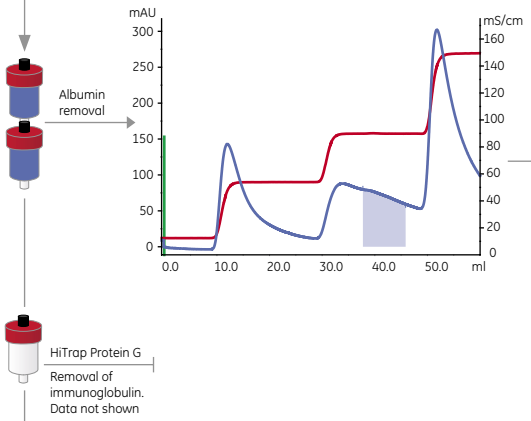


Fig. 16B: HiTrap Blue HP (2 x 5 ml in series)

Sample: 3 ml ISTH/SSC human plasma, secondary coagulation standard, desalted pool
Column: HiTrap Blue HP (2 x 5 ml in series)
Binding buffer: 20 mM Tris-HCl, 100 mM NaCl, pH 7.4
Elution buffer: 20 mM Tris-HCl, 1.0 M NaCl, pH 7.4
Flow rate: 3 ml/min
System: ÄKTAFPLC



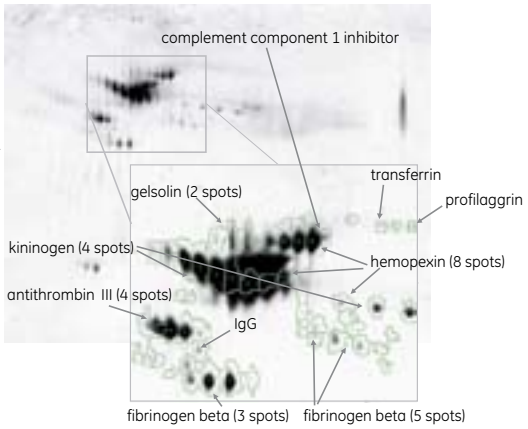
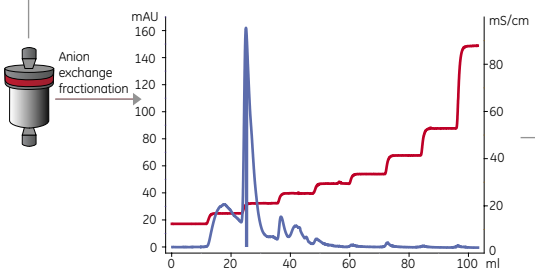
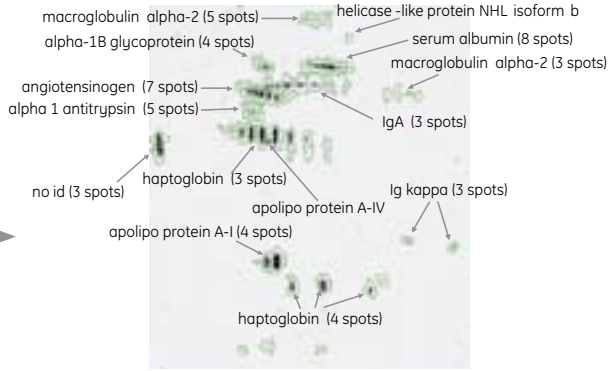


Fig. 16C: RESOURCE Q

Sample: 3 ml ISTH/SSC human plasma, secondary coagulation standard, albumin and immunoglobin removed pool (Figs. 13A and 13B)
Column: RESOURCE Q 5 ml
Start buffer: 20 mM Tris-HCl, 100 mM NaCl, pH 7.4
Elution buffer: 20 mM Tris-HCl, 1.0 M NaCl, pH 7.4
Flow rate: 3 ml/min
Gradient: Segmented
System: ÄKTAFPLC





Conclusions

- Protein concentrations in plasma range from low femtomolar or less and up to millimolar, making analysis of the low abundance proteins extremely challenging
- Chromatographic prefractionation of plasma improves the ability to detect and identify low-abundance proteins in two-dimensional gel electrophoresis and/or liquid chromatography analysis of plasma, followed by MS analysis
- A rapid, simple and semi-automated chromatographic method to fractionate plasma proteins
- The identification of a number of selected proteins is illustrated here, demonstrating the method's simplicity and robustness

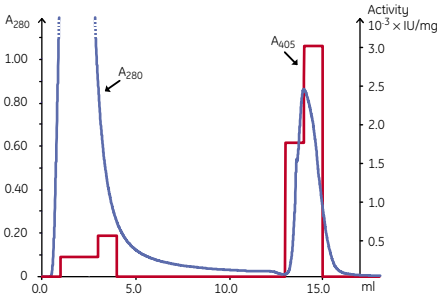
About HiTrap Blue HP

- HiTrap Blue HP is prepacked with Blue Sepharose HP, with the ligand Cibacron™ Blue F3G-A
- Its ligand is covalently attached to the highly cross-linked agarose medium and is stable over a wide pH range
- For purifying albumin, enzymes, coagulation factors, interferons, and related proteins

Removal of trypsin-like serine proteases from human plasma

Fig 17: Removal of trypsin-like serine proteases

Sample: 1 ml human plasma filtered through a 0.45 µm filter
Column: HiTrap Benzamidine FF (high sub), 1 ml
Binding buffer: 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4
Elution buffer: 50 mM glycine, pH 3.0 0-100%
Gradient: elution buffer in one step
Flow rate: 1.0 ml/min
System: ÄKTAexplorer 10



Conclusions

- Proteases included in human plasma can damage the sample if not removed
- Almost all trypsin-like serine protease activity was removed from the human plasma sample and bound to the column

About HiTrap Benzamidine FF (high sub)

- For removal and/or purification serine proteases in one step
- High binding capacity
- Effective removal of thrombin and factor Xa after tag cleavage of recombinant proteins

Small scale-up using buffer exchange

Fig 18A: Five HiTrap Desalting columns connected in series

Sample: 2 mg/ml BSA in 50 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0
Sample vol.: 28% of column volume (1.4, 4.3 and 7.1 ml respectively)
Flow rate: 5 ml/min
Column: HiTrap Desalting, 1 × 5 ml, 3 × 5 ml, 5 × 5 ml
Buffer: 50 mM sodium phosphate, 0.15 M sodium chloride, pH 7.0
System: ÄKTAFPLC

Fig. 18B: HiTrap Desalting 1X5 ml in series

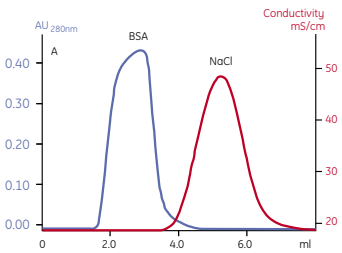


Fig. 18C: HiTrap Desalting 3X5 ml in series

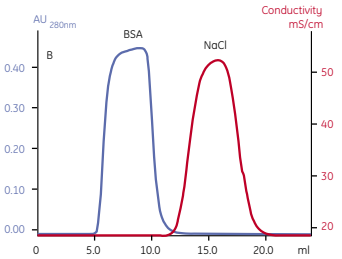
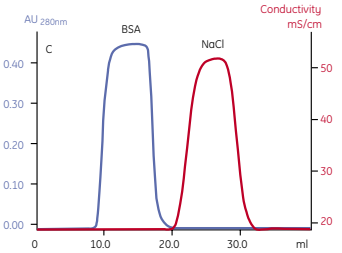


Fig. 18D: HiTrap Desalting 5X5 ml in series



Conclusions

Fig. 18B–D shows results using HiTrap Desalting columns from sample volumes of 1.4, 4.3 and 7.1 mL. Connect HiTrap Desalting columns in series for fast and simple scale-up.

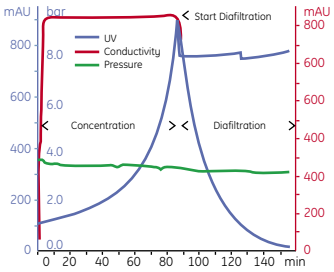
About HiTrap Desalting and HiPrep Desalting

HiTrap Desalting and HiPrep Desalting are prepacked with Sephadex G-25 Superfine for fast, simple desalting and buffer exchange. Using four HiPrep 26/10 Desalting in series yields 60 ml of sample in a very short time.

Desalting using cross-flow filtration for 100-300 ml sample volumes

Fig 19: Desalting of 100 ml using cross-flow filtration

Sample: 100 ml protein solution (1 mg/ml) in 2 M NaCl
Diafiltration buffer: Water
Filtration module: MidGee™ UFP-3-C-MM06A (Filtration area 26 cm²)
Flow Rate: 30 ml/min
System: ÄKTAprime



Conclusions

- Desalting of 100 ml protein solution using cross-flow filtration reduced the salt concentration from 125 mS/cm to 2 mS/cm and the sample was concentrated by a factor of ten
- Cross-flow filtration is a simple purification technique for buffer exchange and desalting

Desalting using cross-flow filtration cartridges

Cross-flow filtration is performed on a MidGee UFP-3-C-MM06A cartridge connected to ÄKTAprime chromatography system.

- Ideal for processing 100-300 ml of feedstock
- Reusable filters deliver reproducible results

Removal of high abundance proteins

Prepacked columns	Quantity	Code no.
HiTrap Blue HP	1 × 5 ml	17-0413-01
HiTrap Blue HP	5 × 1 ml	17-0412-01
RESOURCE Q	1 × 6 ml	17-1179-01
RESOURCE Q	1 × 1 ml	17-1177-01
HiTrap Protein G HP	2 × 1 ml	17-0404-03
HiTrap Protein G HP	1 × 5 ml	17-0405-01
HiTrap Protein G HP	5 × 1 ml	17-0404-01

HiTrap Blue

Removal of trypsin-like serine proteases

Prepacked columns	Quantity	Code no.
HiTrap Benzamidine FF	2 × 1 ml	17-5143-02
HiTrap Benzamidine FF	5 × 1 ml	17-5143-01
HiTrap Benzamidine FF	1 × 5 ml	17-5144-01
Benzamidine Sepharose 4 FF	25 ml	17-5123-10

HiTrap Benzamidine

Desalting and scale up for buffer exchange

Prepacked columns	Quantity	Code no.
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01

Desalting using cross-flow filtration

Membrane filters	Cut-off	Code no.
MidGee UFP-3-C-MM06A	3 kD	56-4100-05
MidGee UFP-10-C-MM06A	10 kD	56-4100-13
MidGee UFP-30-C-MM06A	30 kD	56-4100-21
MidGee UFP-50-C-MM06A	50 kD	56-4100-29
MidGee UFP-100-C-MM06A	100 kD	56-4100-37

MidGee cross-flow cartridge

Automated chromatography system(s) cited

Systems and software	Quantity	Code no.
ÄKTA$_{FPLC}$	1	18-1118-67
ÄKTAprime	1	18-1139-47

Related literature

Selection Guide	
Affinity chromatography columns and media	18-1121-86
Gel filtration column and media	18-1124-19
HiTrap columns	18-1129-81
Hollow fiber cartridges and systems	18-1165-29

Chromatography systems

ÄKTAprime:
simple automated purification

ÄKTA$_{FPLC}$:
high performance purification of proteins & other biomolecules

ÄKTApurifier:
high performance purification and characterization

ÄKTAexplorer:
for fast method development and scale-up

ÄKTApilot:
rapid process development and pilot-scale

ÄKTAxpress:
for high throughput tagged protein purification

Way of working	ÄKTAprime	ÄKTA$_{FPLC}$	ÄKTApurifier	ÄKTAexplorer	ÄKTApilot	ÄKTAxpress
Simple, one-step purification	•	•	•	•	•	
Reproducible performance for routine purification	•	•	•	•	•	•
Optimization of one-step purification to increase purity	•	•	•	•	•	
System control and data handling for regulatory requirements, e.g. GLP		•	•	•	•	
Automatic method development and optimization			•	•	•	
Automatic buffer preparation			•	•		
Automatic pH scouting			•	•		
Automatic media or column scouting				•	•	
Automatic multistep purification				•	•	•
Method development and scale-up				•	•	
Sanitary design cGMP					•	
Scale-up, process development, and transfer to production					•	
Fully-automated, high-throughput, unattended operations						•

UNICORN control system: one software delivers real-time control over all of our chromatography systems. It offers easy-to-use, editable, method wizards for all major applications and techniques. UNICORN enables direct method transfer while providing powerful data reporting and evaluation.

Faster, simpler and more reliable purification. For >50 years.

The Protein Separations business area of GE Healthcare has over 230 R&D staff – a high proportion of whom are chemists, biochemists and engineers. Together with our sales and applications specialists they engage in close collaborations with customers and other global leaders within the life science, biotech and biopharmaceutical industries. With a 50 year proven track record of pioneering and improving most of the techniques used in protein purification, we remain committed to making your applications faster, simpler more reliable and productive. Two recent examples include:

- ÄKTAxpress purification system, which automates multi-step purification on up to 48 samples and delivers up to 50 mg of >95% pure tagged proteins overnight
- Ni Sepharose HP and FF media, available in bulk as well as prepacked in HisTrap and HisPrep columns. This latter combination offers one-step simplicity and up to four times higher binding capacity for purfying histidine-tagged proteins.

High reproducibility. Quality control.

We have the world's largest installed capacity for production of chromatography media, with an annual production capacity of 450 000 liters and/or kilograms. Our media and columns are produced according to validated methods and are strictly quality control-tested. This ensures high batch-to-batch reproducibility of our media and prepacked columns while helping you achieve reproducible results.

Quality assurances.

All our products are manufactured and delivered in accordance to ISO 9001 and are backed up with technical data. Critical materials used to construct equipment (i.e. columns, systems, etc.) are tested for biological safety in accordance with relevant standards. We offer full tracability of all material sources. Plus, we invest in media characterization technologies that prove our media meet their specifications and thereby offer you total reassurance.


Coupling
Linker
Linker
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Ready to support your research

Your local Protein Separations specialists offer you powerful resources. In fact, every minute of every working day they help customers solve purification challenges. Our sales professionals are always available to assist you. You can contact our technical support scientists either online or over the phone. Our distribution professionals as well as our inventory management systems and procedures ensure timely deliveries. Our service professionals provide standard and customized agreements to support equipment and process uptime.

Scientific forums

We establish environments for professionals to share experience and knowledge. Online, we offer education centers and users clubs. We hold or are active in thousands of face-to-face discussion forums every year.

Courses and training

Boosting the knowledge and efficiency levels of your teams offers you a lasting competitive advantage. We offer both general and customized training to address your specific needs. On average, over 450 engineers and scientists attend our Fast Trak™ courses every year.

Technical support literature

Many university departments around the world use our technique handbooks as educational supplements. We support you with technical literature, including product catalogs, scientific posters, application notes and more. These are regularly updated to reflect current developments and are available at: www.chromatography.amershambiosciences.com

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For further reading

Handbook	Code No.
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Recombinant protein purification	18-1142-75
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