GE Healthcare

Pure simplicity for tagged proteins

Contents

The journey from a target gene to purified protein 3

GST-tagged proteins

- Introduction to the GST fusion system 4
- pGEX vectors 5
- Applications 6
- Tag cleavage enzymes 13
- Applications 14
- Detection of GST-tagged proteins 17
- Ordering information 19

Histidine-tagged proteins

- Introduction to histidine-tagged proteins 20
- Applications 21
- Ordering information 31

Strep-tag II proteins

- Introduction to *Strep*-tag II proteins 32
- Applications 33
- Ordering information 36

MBP-tagged proteins

- Introduction to MBP-tagged proteins 38
- Applications 39
- Ordering information 43

Dual-tagged proteins

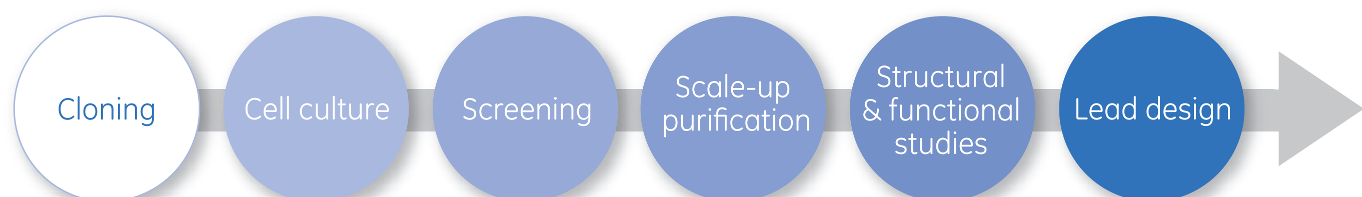
- Introduction to dual-tagged proteins 44
- Applications 45

The journey from a target gene to purified protein

The use of recombinant proteins has increased greatly in recent years, as has the wealth of techniques and products used for their amplification and purification. The advantages of using a recombinant tagged protein to facilitate purification and detection are now widely recognized. In general, the first step in a protein expression workflow involves cloning of the target gene into an appropriate expression vector. This is followed by the transformation of the expression vector into a suitable host system for subsequent expression analysis.

Many host systems are available including bacteria, yeast, plants, filamentous fungi, insect or mammalian cells grown in culture, and transgenic animals or plants. Each host system has its own advantages and disadvantages, and it is important to consider these before the final selection of a host. After initial screening and identification of optimum expression conditions for your particular protein, you can scale-up the purification process to obtain large amounts of the target protein for downstream applications such as functional and structural studies.

The purification of tagged proteins is relatively simple and saves time due to the 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. Further purification steps may be necessary if you require greater purity.



GST-tagged proteins

Glutathione S-transferase (GST) Gene Fusion System is a versatile system for the 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™ chromatography media, resulting in greater than 90% purity in one step of the eluted target molecule. The GST tag may also increase the solubility and stability of the target protein. GST is relatively large with a relative molecular mass (M_r) of 26 000. A specific cleavage sequence allows simple removal of the GST tag with the use of different proteases after purification.

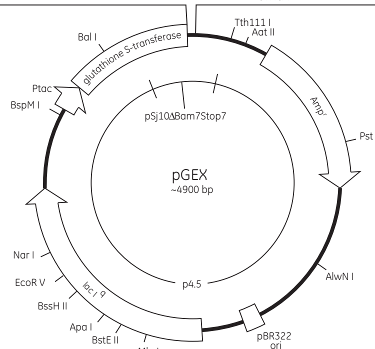
Purification of GST-tagged proteins can be performed under very mild conditions, which preserves the function and antigenicity of the target protein. GST tags are often used to complement histidine tags or as an alternative when histidine tags do not give a soluble protein during expression.

pGEX vectors produce high yields of tagged proteins

GST Gene Fusion System incorporates pGEX plasmids for inducible, high-level intracellular expression of genes or gene-fragments as fusions with *Schistosoma japonicum* GST. Expression in *E. coli* yields tagged proteins in the cytoplasm with the GST moiety at the amino terminus and the protein of interest at the carboxyl terminus.

Thirteen pGEX vectors are available. Nine of the vectors have an expanded multiple cloning site (MCS) that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors.

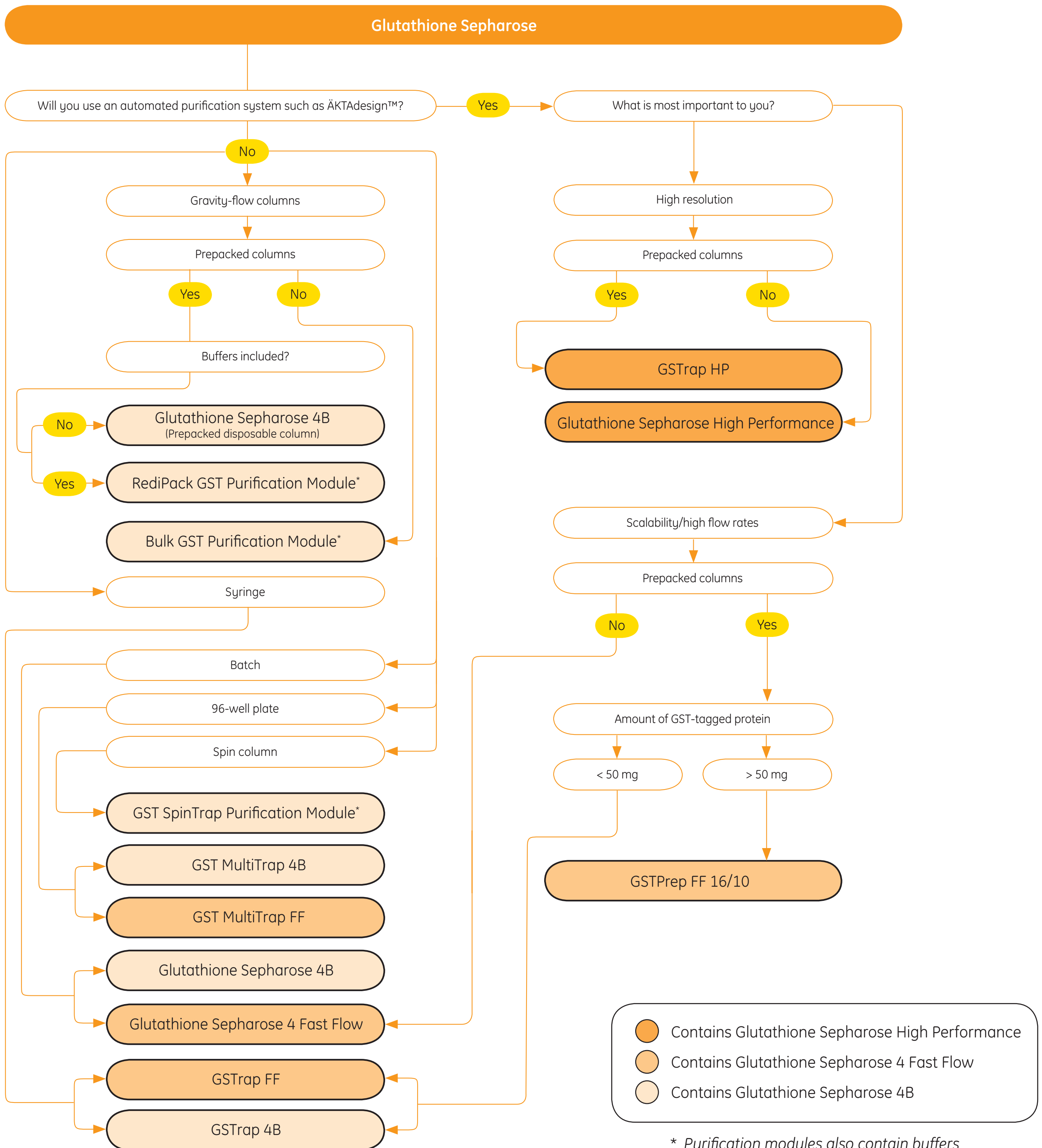
GE Healthcare offers pGEX vectors with encoded recognition sequences for site-specific cleavage by PreScission™ Protease, Thrombin protease, and Factor Xa protease (see pGEX vector sequence map on this page).



Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.

Purification of GST-tagged proteins

Different Glutathione Sepharose chromatography media are available in serveral formats. The media vary in their performance parameters, and the different formats provide options for scale and convenience.



* *Purification modules also contain buffers*

Screening optimal conditions for binding

Products featured: GST MultiTrap FF and GST MultiTrap 4B, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

Proteins are expressed for functional and structural studies and one part of the early screening phase is to find out optimal conditions for binding and elution of the target protein.

The effect of different incubation times for the binding of a GST-tagged protein sample was investigated to achieve the best yield on GST MultiTrap™ FF and GST MultiTrap 4B 96-well filter plates. *E. coli* containing GST-hippocalcin was chemically lysed and sonicated prior to application of the unclarified sample directly to the wells.

96-well filter plates: GST MultiTrap FF and GST MultiTrap 4B

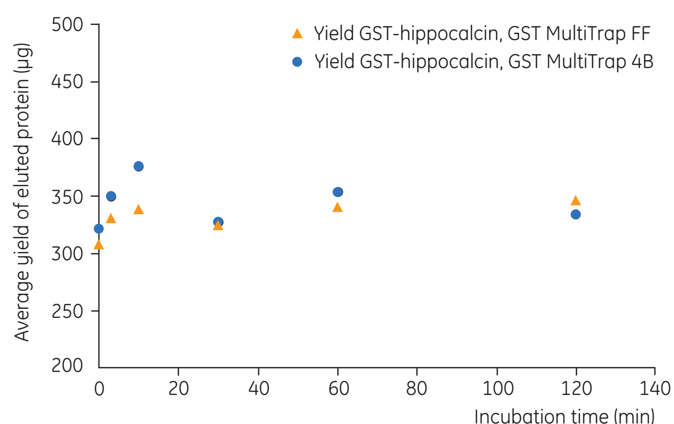
Sample: 300 µl of *E. coli* BL21 lysate containing GST-tagged hippocalcin, M_r 45 000

Sample preparation: Chemical lysis and sonication

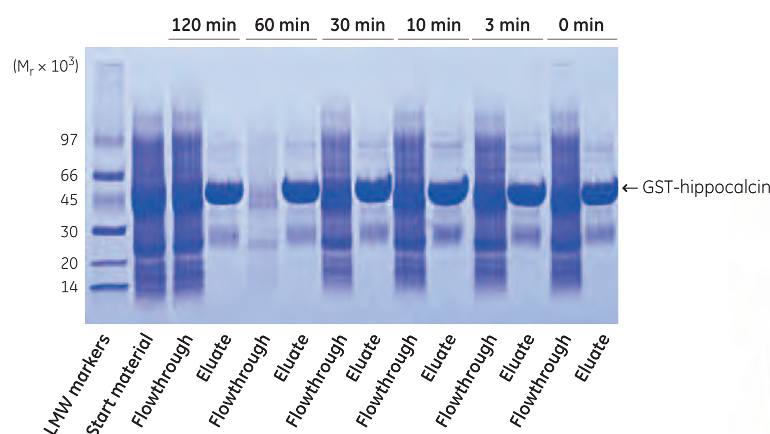
Binding buffer: 10 mM sodium phosphate, 140 mM NaCl, pH 8.0

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

Elution method: Centrifugation



SDS-PAGE



Summary

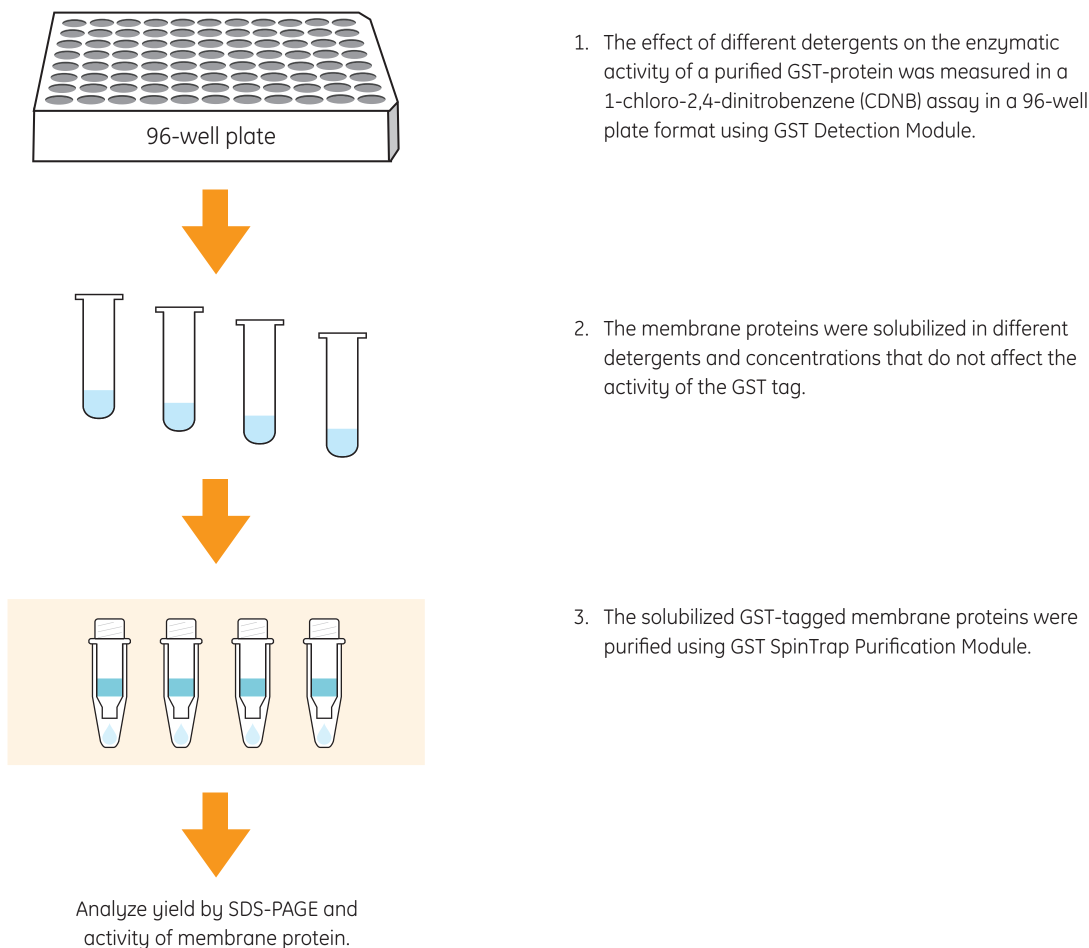
The optimum time determined for the binding of GST-hippocalcin was 3 to 15 min with both GST MultiTrap FF and GST MultiTrap 4B. The yield was ~350 µg using both 96-well plates.

Solubilization screening strategies for GST-tagged membrane proteins

Products featured: GST SpinTrap Purification Module, GST Detection Module

Solubilization is one of the most critical stages of the extraction of membrane proteins. Effective solubilization ensures high yields of biologically active membrane protein and paves the way for successful purification. For effective solubilization of a target membrane protein, it is often necessary to screen several detergents before selecting the best detergent for a particular target protein and its purification strategy.

GST SpinTrap™ columns were used for rapid, simultaneous screening of six different detergents for a GST-membrane protein (GST-ecoKch).

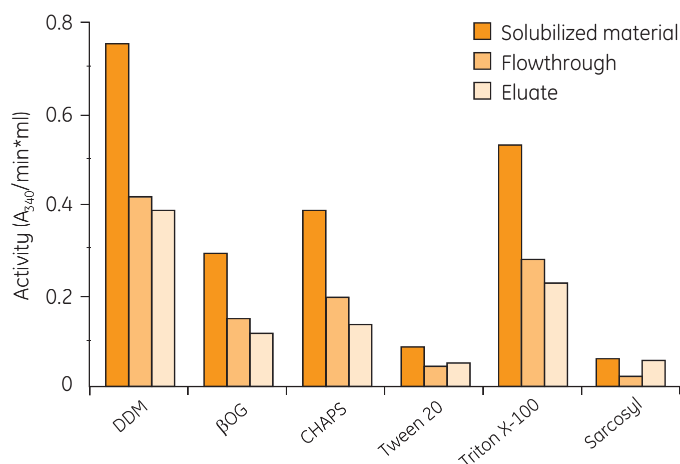


Column: GST SpinTrap

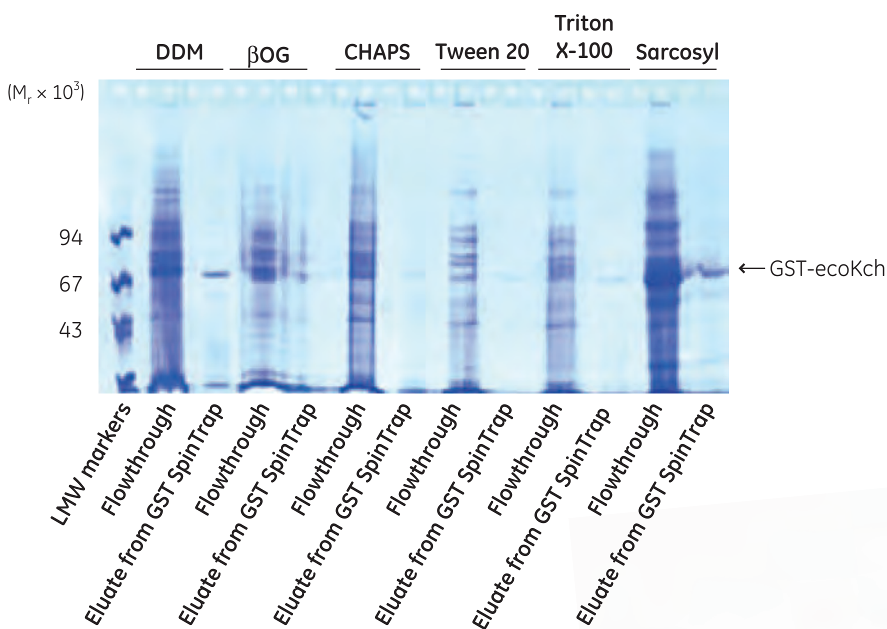
Sample: 500 µl of GST-tagged ecoKch solubilized 1:10 (w/v) in 5% DDM, βOG, CHAPS, Tween™ 20, Triton™ X-100, or Sarcosyl

Binding buffer: PBS, pH 7.5

Elution buffer: PBS, 0.2% detergent, 10 mM reduced glutathione, pH 8.0



SDS-PAGE



Acknowledgements: D. Birse, Department of Biochemistry and Biophysics, Stockholm University, Sweden

Summary

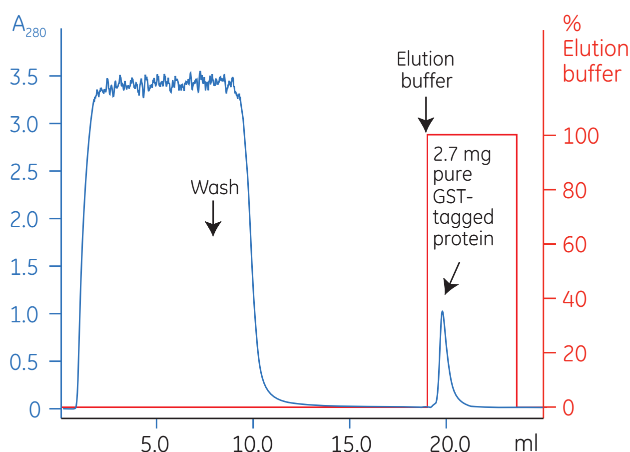
The yield of GST-ecoKch in the solubilization (data not shown) and purification steps was highest with DDM and sarcosyl. Note that sarcosyl greatly decreases GST activity in CDNB assays therefore the results do not reflect the true amounts of GST-tagged protein present. Enzyme activity after purification was highest with DDM. This shows a simple and rapid method for selecting optimum conditions for membrane protein solubilization.

Efficient one-step purification

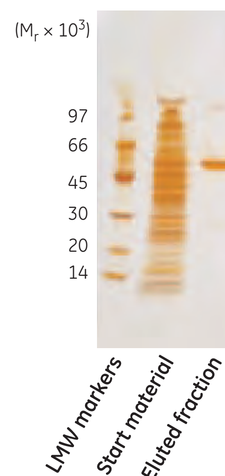
Products featured: GSTrap FF, ÄKTAexplorer, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

Purification of GST-tagged proteins can often be achieved in a single step using convenient prepacked columns combined with a syringe, pump, or chromatography system. In this example, a GSTrap™ FF 1 ml column was used to purify a GST-tagged soluble receptor subunit to a high degree of purity in a single step. A preprogrammed UNICORN™ method template was used in ÄKTAexplorer™ to provide a standard purification protocol that can either be followed exactly or modified to suit your unique requirements. The eluted fraction was analyzed by gel electrophoresis followed by silver staining.

Column: GSTrap FF 1 ml
Sample: 8 ml of cytoplasmic extract from *E. coli* expressing a GST-tagged protein
Binding buffer: PBS, pH 7.3
Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Flow: 1 ml/min
System: ÄKTAexplorer



SDS-PAGE



Summary
GSTrap FF and a preprogrammed method template in ÄKTAexplorer were used to produce highly pure protein in a single purification step.

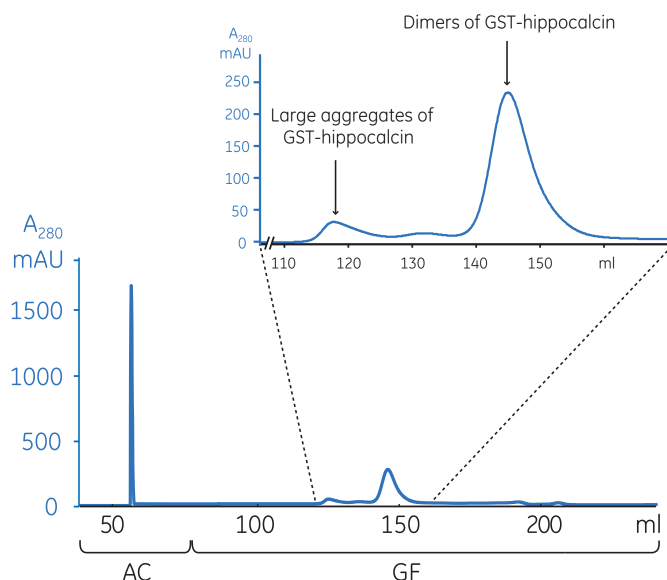
Unattended two-step automated purification

Products featured: GSTrap 4B, HiLoad 16/60 Superdex 200 pg, ÄKTAxpress, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

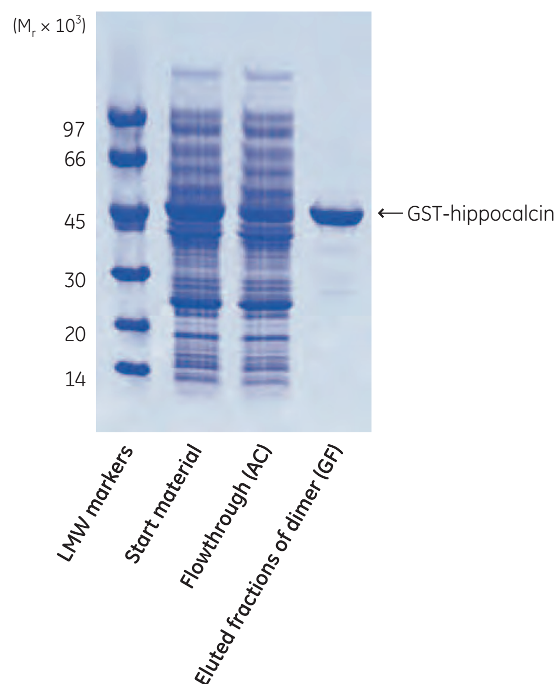
After the initial purification of a GST-tagged protein using Glutathione Sepharose products, free cysteine groups on the GST tag can react with each other to cause aggregates despite the presence of a reducing agent such as dithiothreitol (DDT).

Running a second purification step using gel filtration chromatography provides an efficient method for separating protein aggregates from the active target protein. Additional benefits of such a polishing step include the removal of other contaminants and buffer exchange. ÄKTAxpress™ provides an automated solution for unattended, multistep purification of affinity-tagged proteins. Aggregates of GST-hippocalcin were successfully removed using an automated two-step purification method template in ÄKTAxpress. The two-step method comprised affinity chromatography (AC) and gel filtration (GF) purification. The yield of eluted GST-hippocalcin was measured by absorbance at 280 nm and the purity was analyzed by gel electrophoresis.

Columns: GSTrap 4B 1 ml (AC) and HiLoad™ 16/60 Superdex™ 200 pg, 120 ml (GF)
Sample: Clarified *E. coli* lysate containing expressed GST-hippocalcin, M_r 43 000
Sample volume: 5 ml
Binding buffer (AC): 10 mM sodium phosphate, 140 mM NaCl, 20 mM DTT, pH 7.4
Elution buffer (AC): 50 mM Tris-HCl, 20 mM glutathione, 20 mM DTT, pH 8.0
Buffer (GF): 10 mM sodium phosphate, 140 mM NaCl, 20 mM DTT, pH 7.4
Flow rates: Sample loading, 0.3 ml/min wash and elution, 1 ml/min (AC) 1.5 ml/min (GF)
System: ÄKTAxpress



SDS-PAGE



Summary

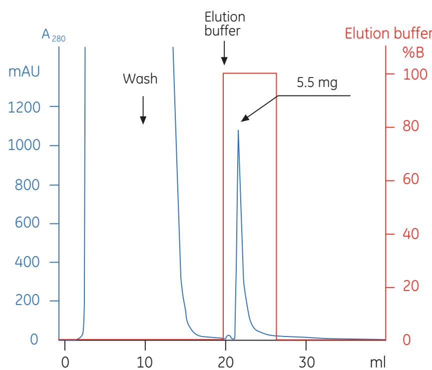
The addition of a second purification step effectively removed aggregates from the GST-hippocalcin sample resulting in the recovery of highly pure target protein.

Increasing the purification scale

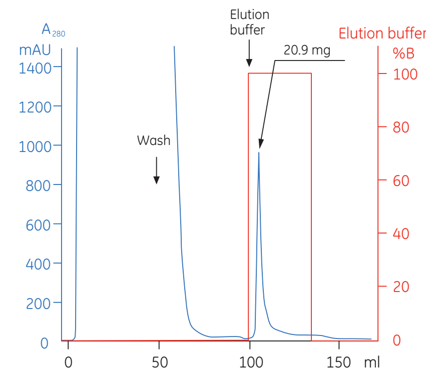
Products featured: pGEX-2T, GSTrap FF, GSTPrep FF 16/10, ÄKTAexplorer, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

Once a method for the purification of a target protein has been established, it can be scaled up to produce larger quantities of target protein for functional and structural studies. 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 is 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. The yield was determined by measuring the absorbance at 280 nm and purity was assessed by SDS-PAGE.

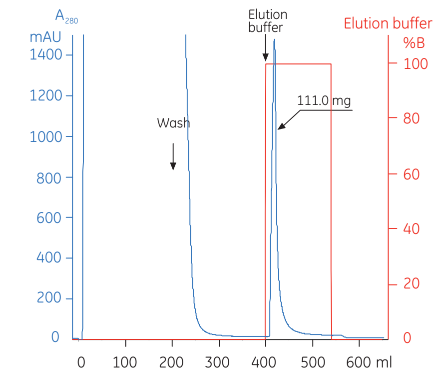
GSTrap FF 1 ml



GSTrap FF 5 ml



GSTPrep FF 16/10



Columns: GSTrap FF 1 ml, GSTrap FF 5 ml, and GSTPrep FF 16/10 (20 ml)

Sample: GST-DemA in *E. coli* extract

Sample volumes: 10 ml (1 ml column); 50 ml (5 ml column), 200 ml (20 ml column)

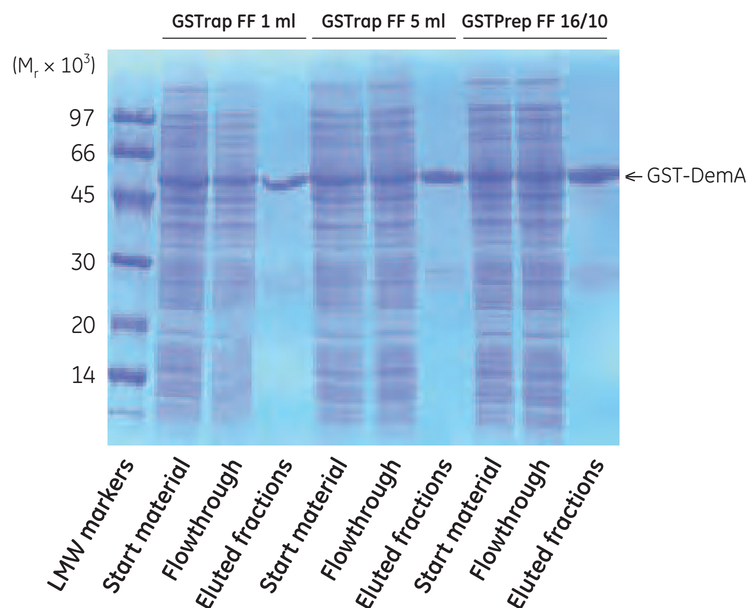
Binding buffer: PBS, pH 7.4

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

Flow rates: 0.5 ml/min at sample loading, 1 ml/min at washing and elution (GSTrap FF 1 ml)
2.5 ml/min at sample loading, 5 ml/min at washing and elution (GSTrap FF 5 ml)
5 ml/min at sample loading, 10 ml/min at washing and elution (GSTPrep FF 16/10)

System: ÄKTAexplorer 100

SDS-PAGE



Summary

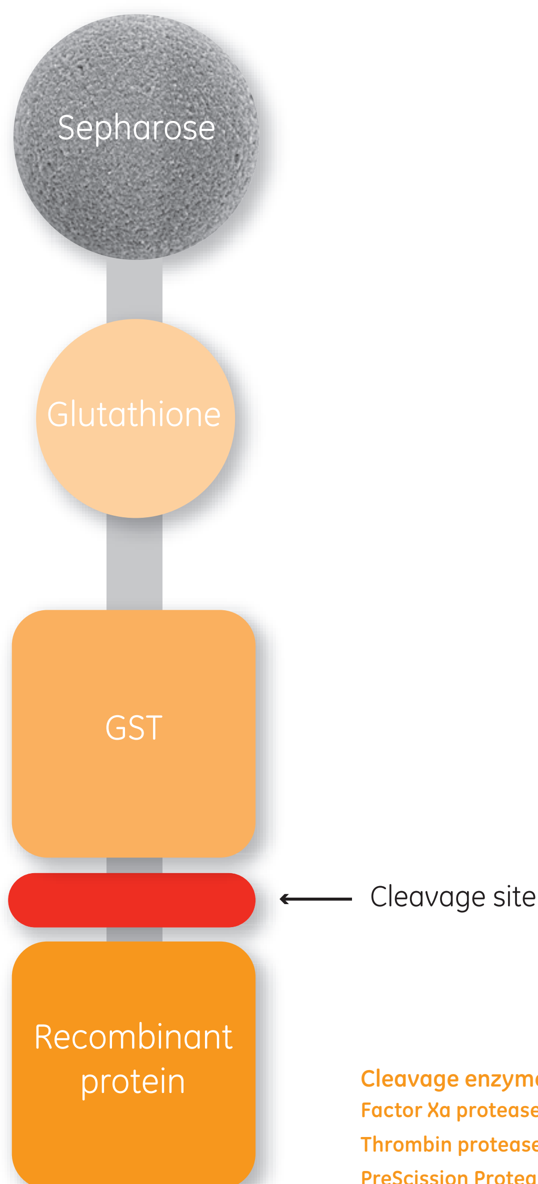
An increased column volume and sample load led to a proportionally increased yield of the eluted GST-tagged protein in the scale-up protocols.

Tag cleavage enzymes

Removal of large protein tags such as the GST tag is often necessary for target protein characterization. The amount of protease, temperature, and length of incubation required for complete digestion varies according to the nature of the particular target protein.

PreScission Protease is an efficient enzyme for the specific cleavage and removal of GST tags. PreScission Protease is by itself a tagged protein of GST and human rhinovirus 3C protease, hence it is easily removed from the cleaved target protein (along with GST) using Glutathione Sepharose affinity medium. Since PreScission Protease is optimally active at 4°C, cleavage can be performed at low temperatures to enhance the stability of the target protein.

Unlike other proteases, the recognition site of PreScission Protease is not a naturally occurring sequence and this confers a high degree of specificity on the enzyme. Cleavage of GST-tagged proteins can also be performed with other proteases that recognize different cleavage sites. Factor Xa protease and Thrombin protease are serine proteases with optimal cleavage performance at room temperature.



Cleavage enzymes

Factor Xa protease: M_r 28 000 - 30 000

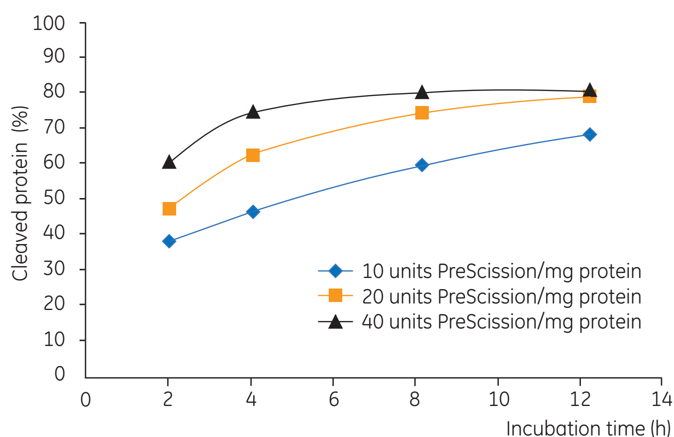
Thrombin protease: M_r 37 000

PreScission Protease: M_r 46 000

Optimization of tag cleavage

Products featured: GSTrap FF, PreScission Protease, ÄKTAxpress

An optimal condition for cleavage was efficiently identified by screening experiments with on-column cleavage of a GST-tagged protein using GSTrap FF and PreScission Protease on an ÄKTAxpress system. Incubation time and the ratios of protease and tagged protein were varied. The percentage of cleaved protein was determined by integrating the area of the the eluted, cleaved protein and dividing this with the integrated total peak area (eluted cleaved protein + uncleaved protein). The cleavage reaction was performed at 4°C.



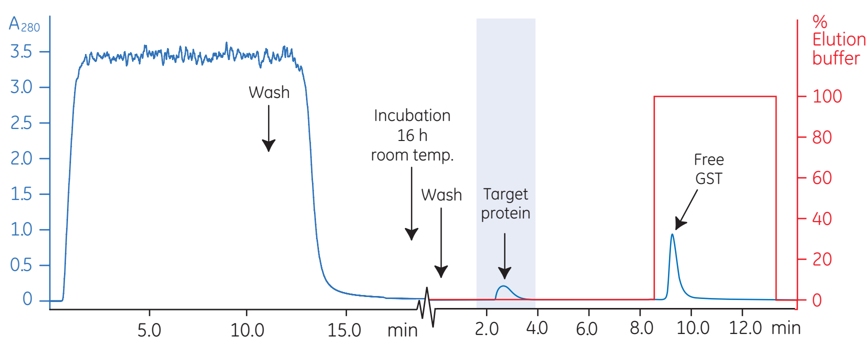
Summary

The optimized conditions identified were 20 U of protease/mg of target protein for a total incubation time of 8 h. This optimal condition was used for further studies.

Purification and on-column cleavage

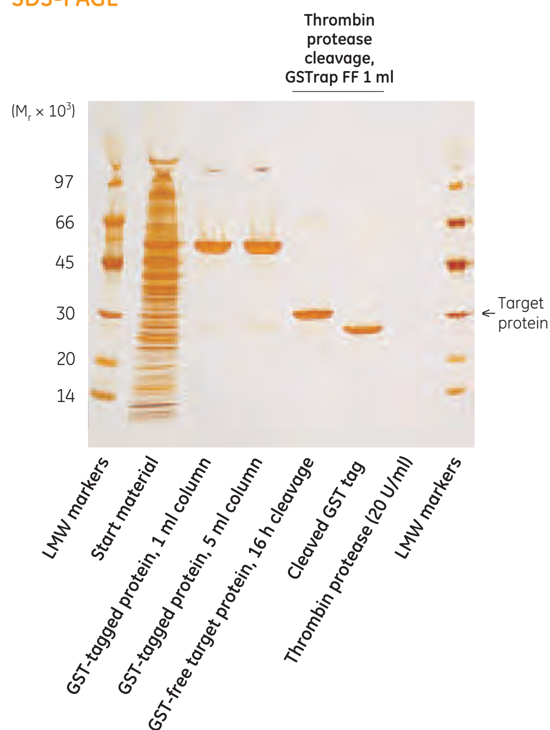
Products featured: GSTrap FF, Thrombin protease, ÄKTAexplorer, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

Cleavage of the GST affinity tag can either be performed on-column before elution or in solution after elution of the target molecule. To demonstrate the efficiency of on-column cleavage in conjunction with purification, a GST-tagged protein containing the recognition sequence for Thrombin protease was applied to a GSTrap FF 1 ml column. After incubation with the protease for 16 h at room temperature, the GST-free target protein was eluted.



Column: GSTrap FF 1 ml
Sample: 10 ml of clarified cytoplasmic extract from *E. coli* expressing a GST-tagged protein
Binding buffer: PBS, pH 7.3
Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Flow rate: 1 ml/min
System: ÄKTAexplorer 10

SDS-PAGE



Summary

Effective on-column cleavage of the GST moiety with Thrombin protease can be integrated with the GST purification process.

Automated multistep purification and tag removal

Products featured: pGEX-6P, GSTrap HP, PreScission Protease, HiLoad 16/60 Superdex 75 pg, ÄKTAxpress, ExcelGel SDS gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

GST-tagged proteins produced with a PreScission Protease cleavage site enable two-step purification with on-column tag cleavage in the first affinity step. A GST-tagged model protein was purified with ÄKTAxpress using an automated two-step purification method. Cleavage and removal of the GST tag was implemented in the protocol.

Columns: Affinity chromatography (AC), GSTrap HP 5 ml, Gel filtration (GF), HiLoad 16/60 Superdex 75 pg

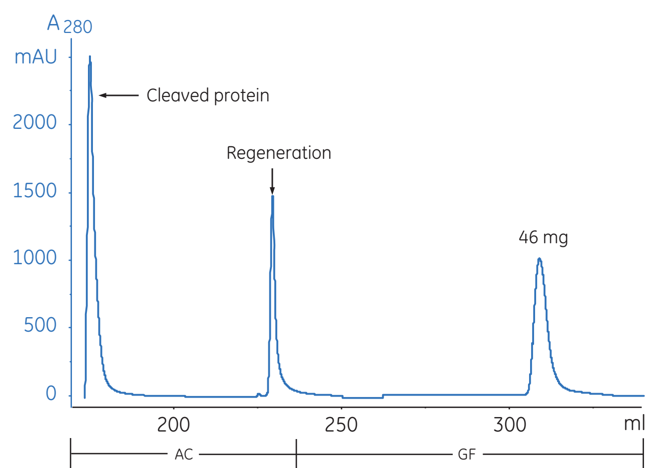
Sample: GST-purα (M_r 61 600)

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

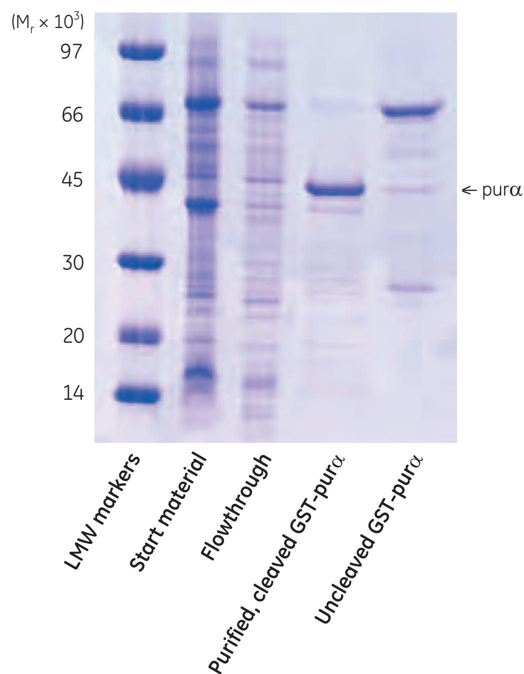
Elution buffer (AC): 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

Buffer (GF): 50 mM Tris-HCl, 150 mM NaCl, pH 7.5

System: ÄKTAxpress



SDS-PAGE



Summary

Tag cleavage and removal were incorporated into the automated two-step purification protocol and this produced a highly pure and cleaved target protein.

Detection of GST-tagged proteins

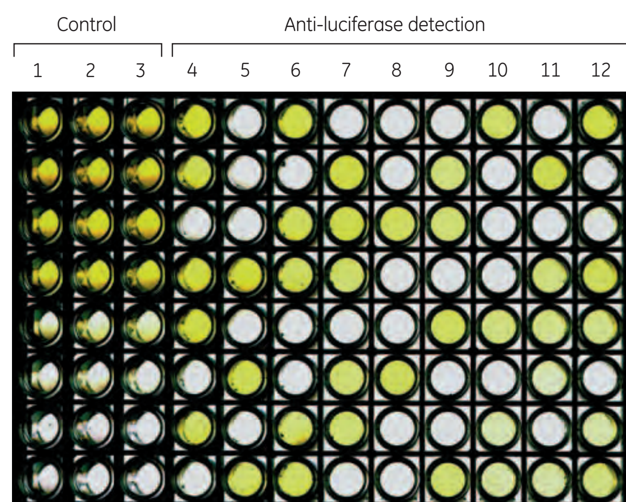
Protein immunodetection is a detection method that measures a specific antigen/antibody reaction. GST 96-well Detection Module makes it possible to visualize and quantitate GST-tagged proteins from a complex protein sample. The way a recombinant protein folds can sometimes mask some of its binding sites during antibody detection of GST-tagged proteins. The use of an anti-GST polyclonal antibody capable of recognizing more than one epitope on GST-tagged proteins greatly enhances the chances of detection.

Identification of clones expressing GST-tagged protein

Products featured: pGEX-6P-1, GST 96-well Detection Module

Once the gene of interest has been cloned into the pGEX expression vector and the host cells used for the cloning step have been transformed, chemically inducible high-level expression of the target protein is possible. The next step is to optimize GST-tagged protein expression and a key step is the capability to screen lysates from many clones.

Cultures of randomly selected *E. coli* colonies resulting from a pGEX-6P-1/luciferase gene cloning experiment were grown, induced, and lysed in a 96-well plate. Captured GST-tagged luciferase detected with rabbit anti-luciferase, anti-rabbit IgG/peroxidase conjugate using GST 96-well Detection Module. TMB (3,3´,5,5´-tetramethyl benzidine) was used as substrate and the absorbance was read at 450 nm.



Columns

1–3 Serial dilutions of control GST-luciferase

4-12 50 µl of clarified lysates from selected colonies

Summary

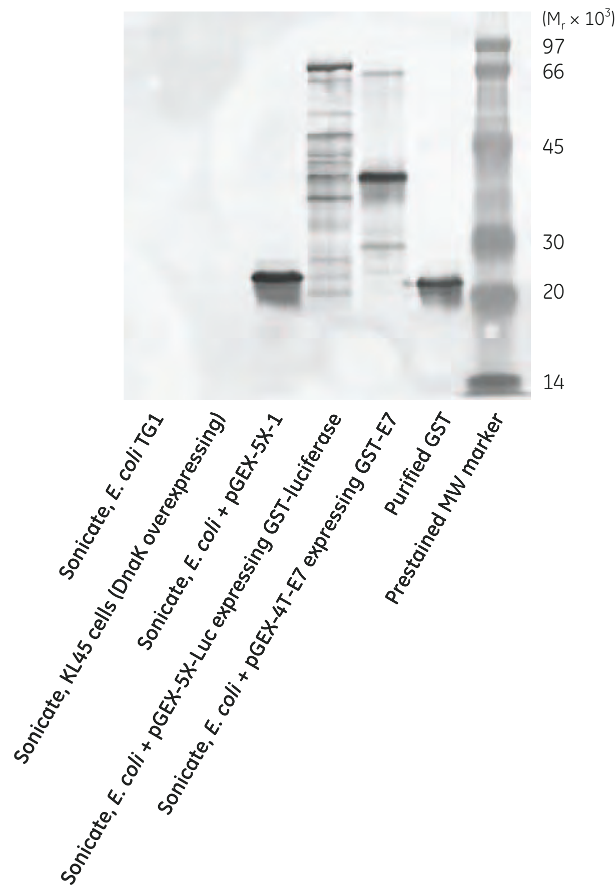
Fifty-three percent of the clones tested expressed GST-tagged luciferase.

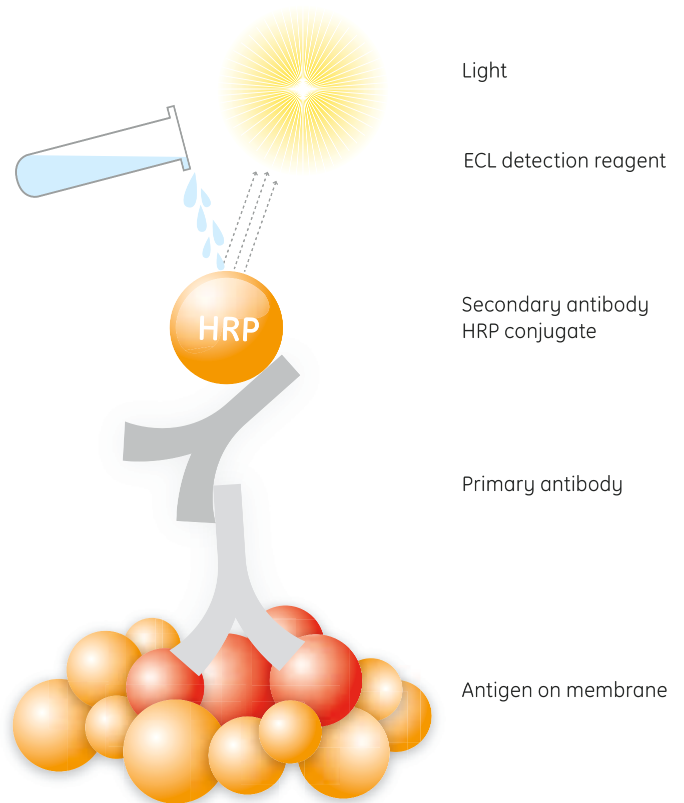
Western blot detection

Products featured: Anti-GST Antibody, Amersham Hybond ECL, Amersham Hyperfilm ECL, Amersham GST Western Blotting Detection Kit

Sometimes, *E. coli* cells may have difficulty overexpressing a particular target protein. To test the efficacy of the expression system, *E. coli* cells containing a pGEX-5X-1 vector without an insert, were induced to overexpress the GST moiety only. Two different GST-tagged proteins were then overexpressed in *E. coli* and detected by the use of Anti-GST Antibody and Western blot using ECL™ detection.

SDS-PAGE





Summary
Anti-GST Antibody provides a quick and convenient method for detecting GST-tagged proteins.

Ordering information

Product	Quantity	Code No.
Protein expression		
pGEX-2T	25 µg	27-4801-01
pGEX-2TK	25 µg	27-4587-01
pGEX- 4T-1	25 µg	27-4580-01
pGEX- 4T-2	25 µg	27-4581-01
pGEX- 4T-3	25 µg	27-4583-01
pGEX-3X	25 µg	27-4803-01
pGEX1λT	5 µg	27-4805-01
pGEX- 5X-1	25 µg	27-4584-01
pGEX- 5X-2	25 µg	27-4585-01
pGEX- 5X-3	25 µg	27-4586-01
pGEX- 6P-1	25 µg	27-4597-01
pGEX- 6P-2	25 µg	27-4598-01
pGEX- 6P-3	25 µg	27-4599-01
All vectors include E. coli BL21		
Purification		
GSTrap HP	5 × 1 ml	17-5281-01
	100 × 1 ml†	17-5281-05
	1 × 5 ml	17-5282-01
	5 × 5 ml	17-5282-02
	100 × 5 ml†	17-5282-05
GSTrap FF	2 × 1 ml	17-5130-02
	5 × 1 ml	17-5130-01
	100 × 1 ml†	17-5130-05
	1 × 5 ml	17-5131-01
	5 × 5 ml	17-5131-02
	100 × 5 ml†	17-5131-05
GSTPrep FF 16/10	1 × 20 ml	17-5234-01
GST MultiTrap FF	4 × 96-well filter plates	28-4055-01
GSTrap 4B	5 × 1 ml	28-4017-45
	100 × 1 ml†	28-4017-46
	1 × 5 ml	28-4017-47
	5 × 5 ml	28-4017-48
	100 × 5 ml†	28-4017-49
GST SpinTrap Purification Module	50 × 50 µl	27-4570-03
GST MultiTrap 4B	4 × 96-well filter plates	28-4055-00
GST Detection Module	50 detections	27-4590-01
GST 96-Well Detection Module	5 plates	27-4592-01

Product	Quantity	Code No.
Detection		
Anti-GST Antibody	0.5 ml, 50 detections	27-4577-01
Amersham ECL GST Western Blotting Detection Kit	1 kit	RPN1237
Amersham Hyperfilm™ ECL (18 x 24 cm)	50 sheets	28-9068-36
Amersham Hybond™ ECL (20 × 20 cm)	10 sheets	RPN2020D
Tag cleavage		
Thrombin protease	500 units	27-0846-01
Factor Xa protease	400 units	27-0849-01
PreScission Protease	500 units	27-0843-01
Related products		
Glutathione Sepharose High Performance	25 ml	17-5279-01
	100 ml	17-5279-02
Glutathione Sepharose 4 Fast Flow	25 ml	17-5132-01
	100 ml	17-5132-02
	500 ml	17-5132-03
Glutathione Sepharose 4B	10 ml	17-0756-01
	100 ml (function tested)	27-4574-01
	300 ml	17-0756-04
Bulk GST Purification Module	1 kit	27-4570-01
RediPack GST Purification Module	1 kit	27-4570-02
Glutathione Sepharose 4B (prepacked disposable columns)	2 x 2 ml	17-0757-01
HiLoad 16/60 Superdex 30 pg	1 × 120 ml	17-1139-01
HiLoad 26/60 Superdex 30 pg	1 × 320 ml	17-1140-01
HiLoad 16/60 Superdex 75 pg	1 × 120 ml	17-1068-01
HiLoad 26/60 Superdex 75 pg	1 × 320 ml	17-1070-01
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 200 pg	1 × 320 ml	17-1071-01
ExcelGel™ SDS Gradient 8-18	6	80-1255-53
LMW-SDS Marker Kit	10 vials	17-0446-01

† available by specific customer order

For more information on equipment for chromatography and/or electrophoresis, please visit www.gelifesciences.com

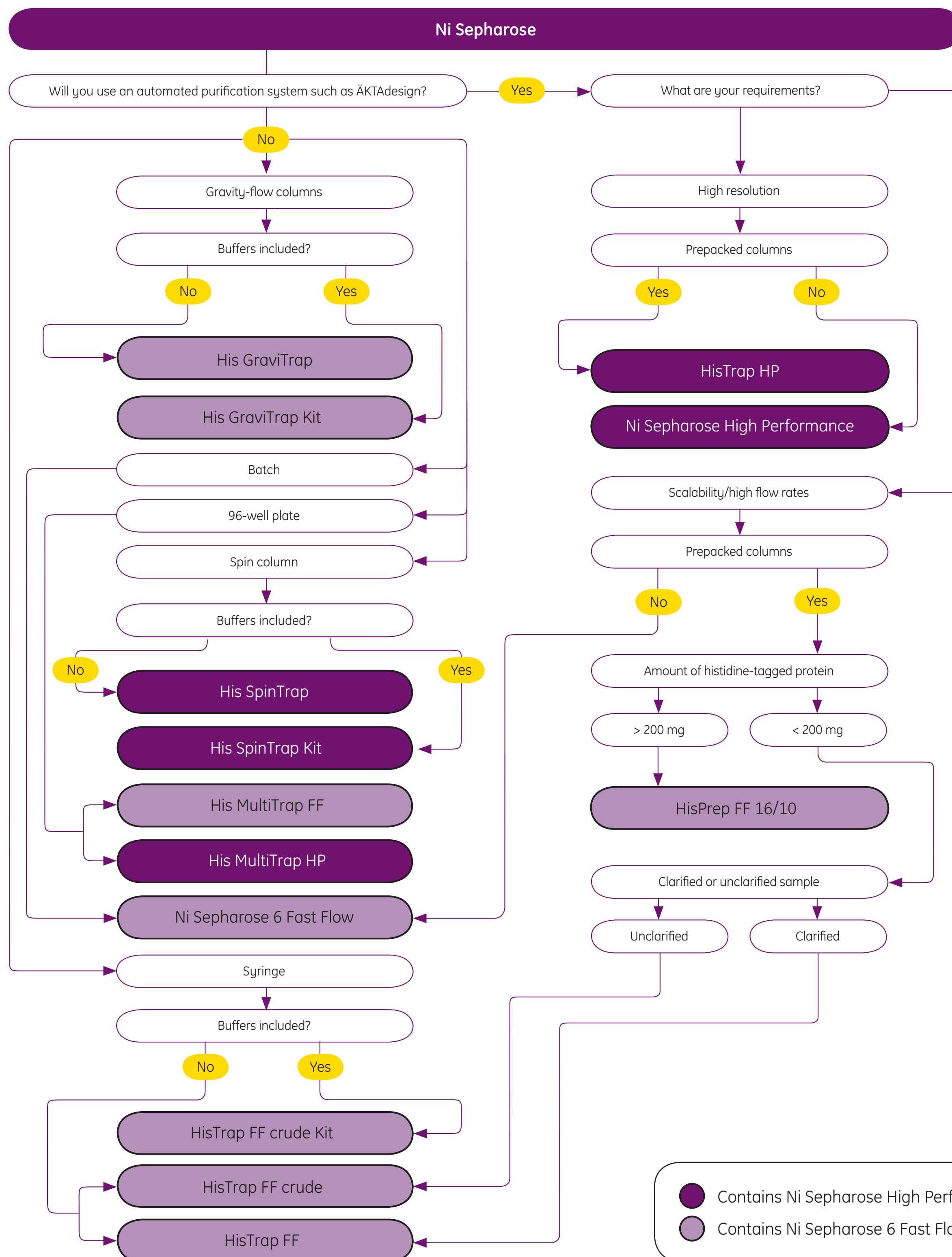
Histidine-tagged proteins

Histidine tags are widely used because 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 chromatography media charged with divalent metal ions such as nickel selectively retain histidine-tagged proteins and allow for 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.

However, since many proteins have intrinsic histidine and/or cysteine amino acid residues, other nonspecific proteins bind to the IMAC media together with the target protein. In such cases, it is often necessary to optimize binding, wash, and elution conditions by varying the concentration of imidazole in these solutions. Increasing the concentration of imidazole in the binding and wash buffers generally decreases nonspecific binding, whereas lower concentrations give stronger affinity interaction. The key is finding the right balance.

Purification of histidine-tagged proteins

Different Ni Sepharose chromatography media are available in serveral formats. The media vary in their performance parameters, and the different formats provide options for scale and convenience.



Automation of high-throughput expression screening

Products featured: His MultiTrap HP

Automated and reproducible protocols for efficient high-throughput purification or expression screening of tagged proteins have become a key step in the search for drug targets. A two-part study was conducted to measure the robustness of an optimized, automated protocol performed in a vacuum on a liquid handling station. First, a chessboard study was performed to measure possible cross-contamination. A histidine-tagged protein sample was applied to every second well and the other wells were left empty. In the second part, the degree of reproducibility was determined by the application of six different histidine-tagged proteins replicated in seven rows. The eluted histidine-tagged proteins were analyzed by SDS-PAGE.

96-well filter plate: His MultiTrap HP

Samples: Chessboard study: Two *E. coli* samples, one expressing a (histidine)$_6$-tagged recombinant protein (M_r 37 000) and a sample where no recombinant protein was expressed.
Reproducibility study: Six different *E. coli* samples expressing different sizes of recombinant proteins (see SDS-PAGE below). Two of the six recombinant proteins (2 and 6) were not expressed.

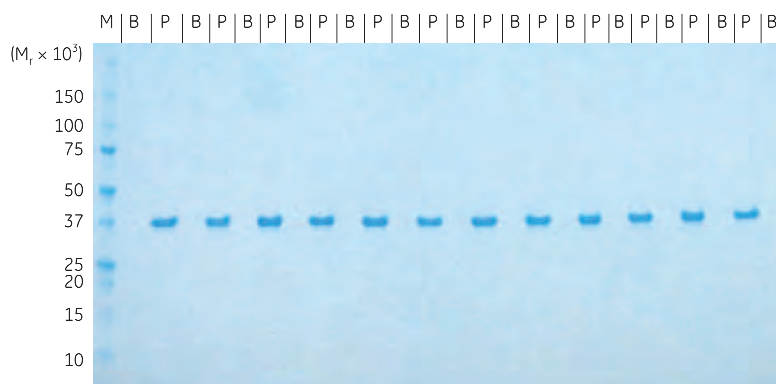
Equilibration buffer: 20 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.4

Wash buffer: 20 mM Tris-HCl, 500 mM NaCl, 25 mM imidazole, pH 7.4

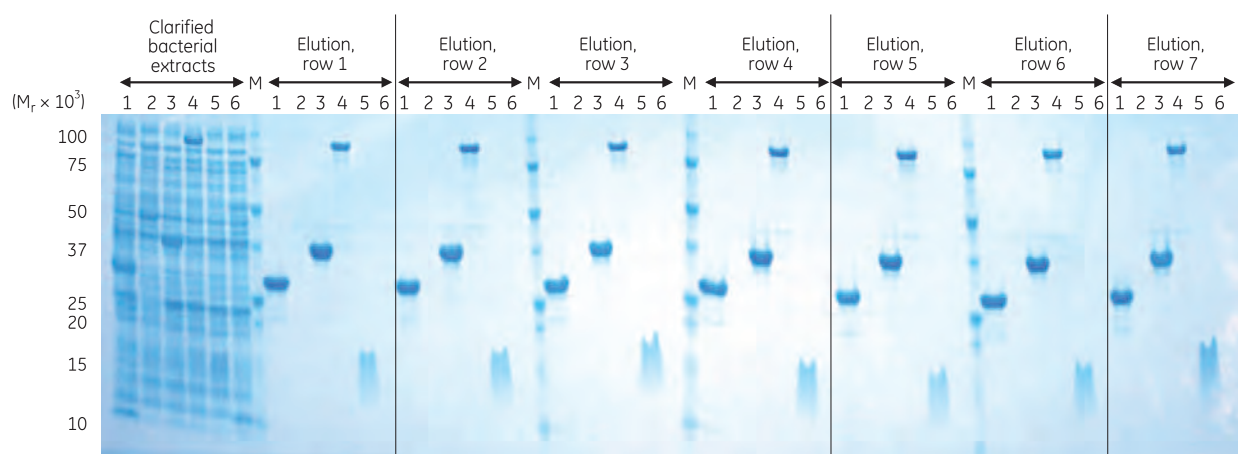
Elution buffer: 20 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.4

Liquid handling station: Hamilton MICROLAB™ STAR

Vacuum station: MICROLAB STAR Basic Vacuum System (BVS)



P = Protein purification (M_r 37 000)
B = Blank



Acknowledgements: B. Gallet, M. Noirclerc-Savoye and T. Vernet, RoBioMol/Laboratory for Macromolecular Engineering, Institut de Biologie Structurale CEA-CNRS-UJF, Grenoble, France

Summary

Automation methods increase throughput at each stage and enable a high degree of robustness with negligible cross contamination and a highly consistent well-to-well performance.

Optimizing purification conditions

Products featured: His SpinTrap, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

The imidazole concentration during binding and washing is an important factor that affects the final purity and yield of the target protein. This was demonstrated by a series of experiments in which a histidine-tagged protein, APB 7-(His)$_6$, (M_r 28 000), was purified on His SpinTrap using 5, 50, 100, or 200 mM imidazole concentrations in the sample and binding buffers.

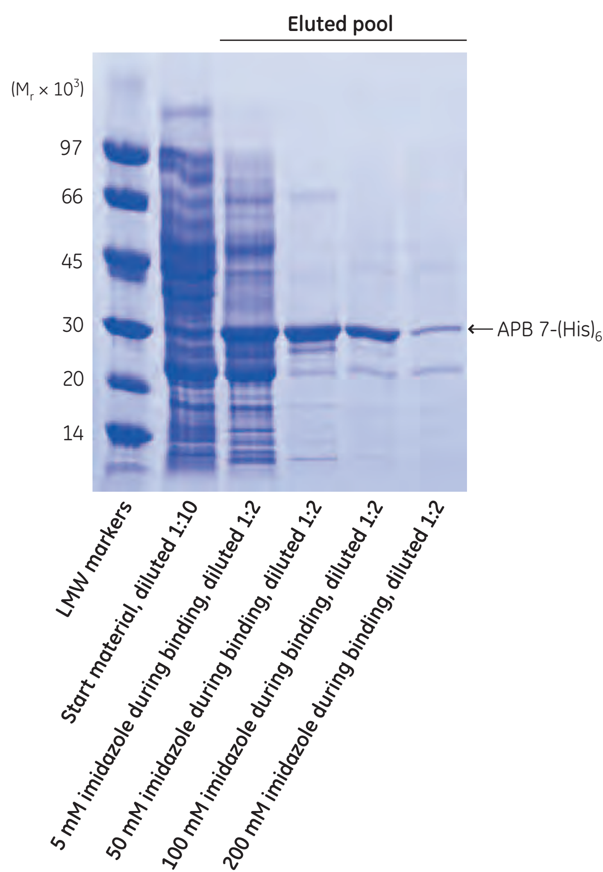
Column: His SpinTrap

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

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

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

SDS-PAGE



Summary

In this application, 50 mM imidazole prevented the binding of most contaminants and improved purity. Adding more imidazole to the sample and binding buffers led to a marginal increase in purity but lower protein yield.

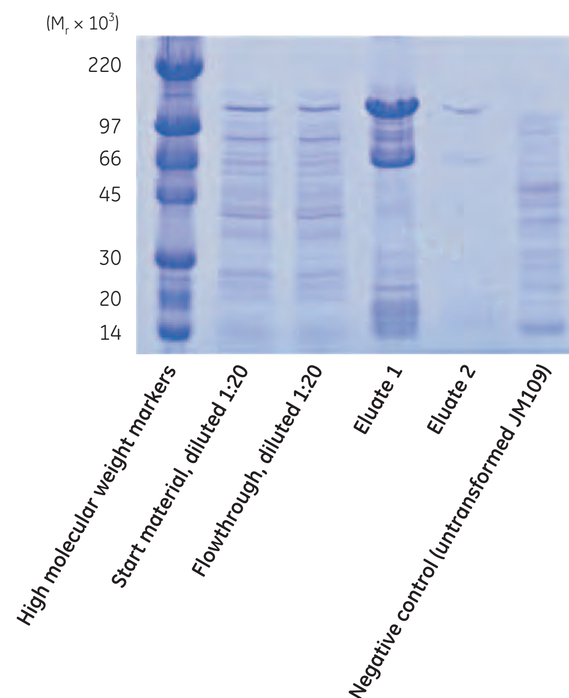
Manual gravity purification

Products featured: His GraviTrap Kit, PhastSystem, PhastGel Gradient 10–15, Amersham Hybond ECL, Anti-His Antibody, ECL Mouse IgG, HRP-linked Whole Ab, Amersham High-Range Rainbow Molecular Weight Markers

The gravity purification method can be effective when you need to purify large samples in the absence of a chromatography system. Direct purification without prior clarification of the bacterial cell lysates can be accomplished by using His GraviTrap™ columns. In this example, a high molecular weight (histidine)$_{10}$-tagged protein was purified in 25 min from 20 ml of clarified *E. coli* JM109 lysate containing TRX-P450-(His)$_{10}$ (M_r~ 130 000). The eluted fractions were analyzed by SDS-PAGE and Western blotting.

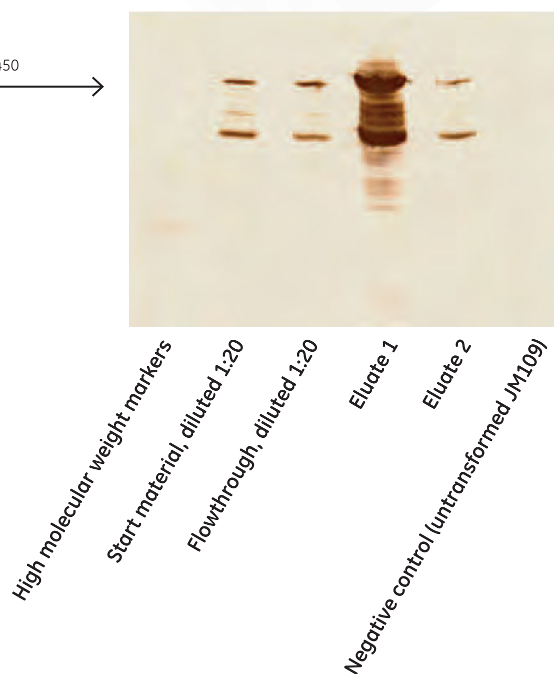
Column: His GraviTrap
Sample: 20 ml clarified *E.coli* JM109 lysate containing (His)$_{10}$-TRX-P450 (M_r ~130 000)
Binding/wash buffer: 20 mM phosphate, 500 mM NaCl, 40 mM imidazole, pH 7.4
Elution buffer: 20 mM phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

SDS-PAGE



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

Western blot



Summary
Both SDS-PAGE and Western blot analyses of the eluted fractions showed three major bands indicating that histidine tags were present. The top band is the targeted full-length protein and the bands below represent truncated forms of the histidine-tagged target protein.

Manual purification using a syringe

Products featured: HisTrap FF crude Kit, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

Direct loading of unclarified cell lysates decreases the total purification time and increases the chances of purifying sensitive target proteins without losing activity. In the absence of a chromatography system, histidine-tagged proteins from unclarified cell lysates can be purified in a matter of minutes using a column and syringe. In this example, convenient and simple purification of a histidine-tagged maltose binding protein from an unclarified sample was performed using a syringe and the buffers included in HisTrap™ FF crude Kit.

Column: HisTrap FF crude 1 ml

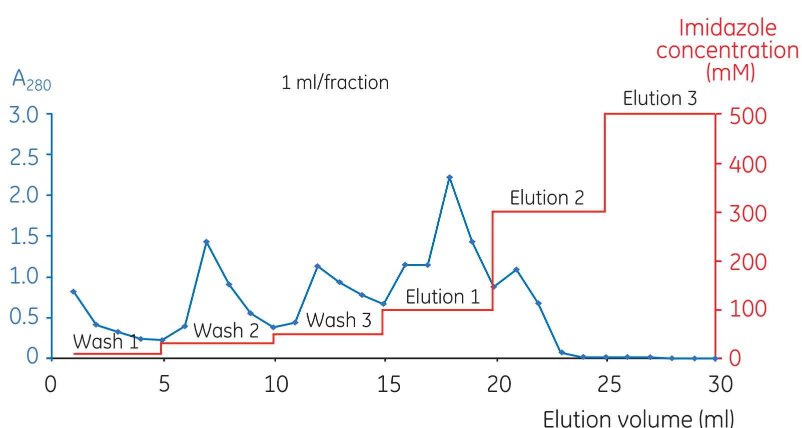
Sample: Unclarified *E. coli* extract containing MBP-(His)$_6$, M_r 43 000

Flow rate: Approx. 1 ml/min

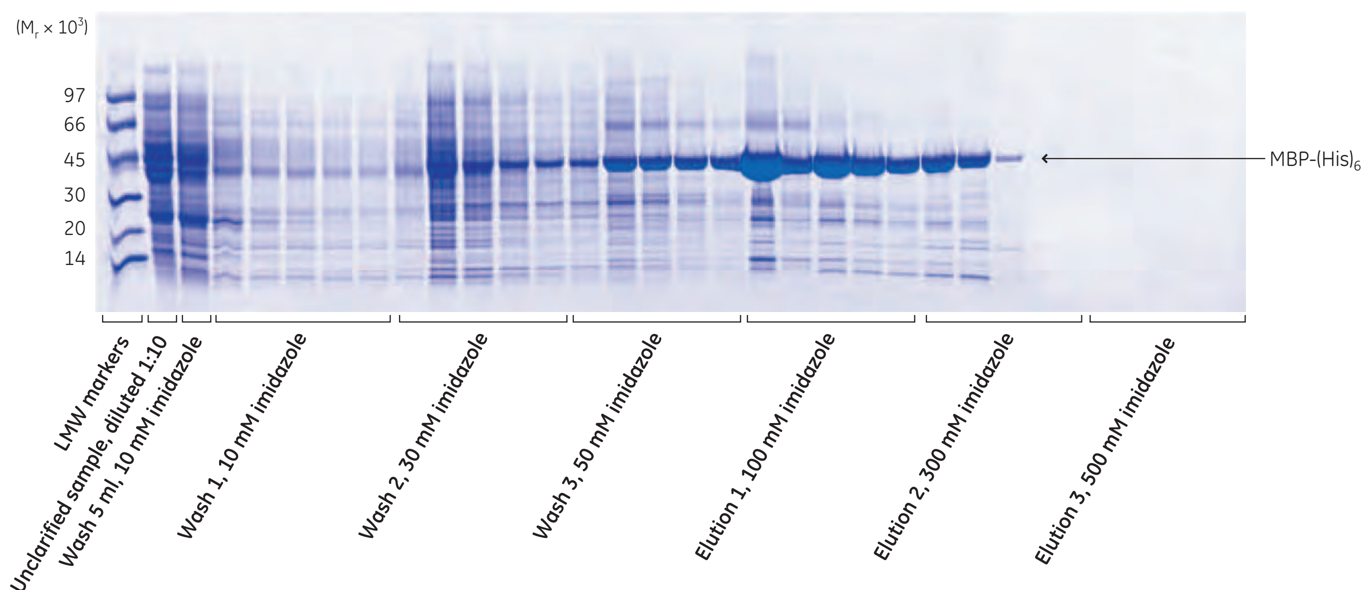
Buffers: Included in the HisTrap FF crude Kit

Fraction size: 1 ml

Equipment: Manual purification using a syringe



SDS-PAGE



Summary

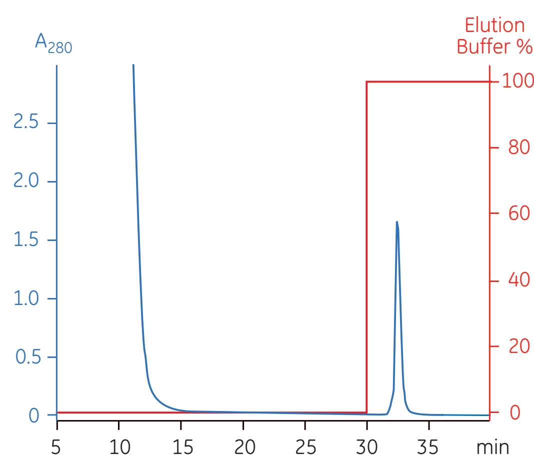
MBP-(His)$_6$ was quickly isolated from an unclarified sample using a syringe.

Simple one-step purification

Products featured: HisTrap HP, ÄKTAprime plus

Highly pure histidine-tagged proteins can be obtained in a single purification step with a chromatography system. In the example shown, a step-gradient elution was used to efficiently purify a histidine-tagged protein. The purification process was simplified by the use of optimized protocols on an ÄKTAprime™ plus.

Sample: Clarified homogenate of *E. coli* expressing histidine-tagged protein
Column: HisTrap HP 1 ml
Binding buffer: 20 mM phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4
Elution buffer: 20 mM phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4
System: ÄKTAprime plus

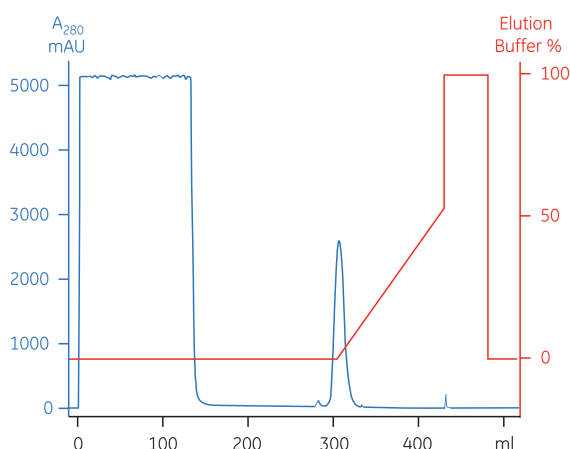


Summary
Efficient purification using step elution was achieved in 35 min.

Purification of a histidine-tagged protein expressed in *Pichia pastoris*

Products featured: HisTrap FF crude, ÄKTAexplorer, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

Even in cases where the target histidine-tagged protein is expressed at very low levels, it is possible to obtain relatively large quantities of pure protein because the presence of the histidine tag effectively enriches the target protein on the affinity column. An unclarified lysate of a histidine-tagged protein expressed in *Pichia pastoris* was loaded directly onto a column. The protein was eluted using a linear gradient.



Column: HisTrap FF crude 5 ml

Sample: 130 ml of unclarified lysate of YNR064c (*Saccharomyces cerevisiae* hydrolase) expressed in *P. pastoris*

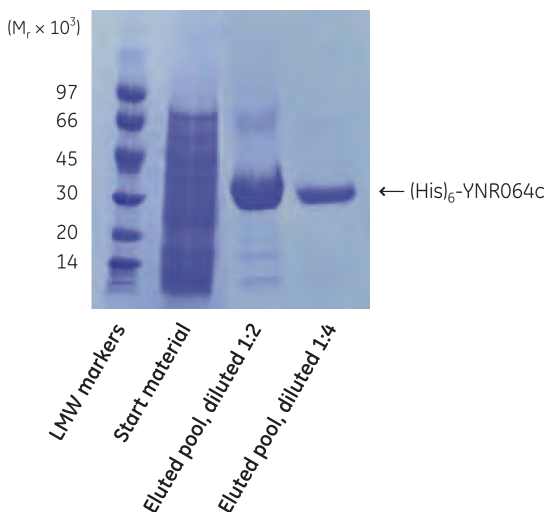
Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 75 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, 3 mM KCl, pH 7.4

Flow rate: 5 ml/min

System: ÄKTAexplorer 100

SDS-PAGE



Summary
A highly pure target protein was obtained from an unclarified yeast lysate using gradient elution.

One-step purification combined with on-column refolding

Products featured: HisTrap HP, ÄKTAexplorer, Biacore System

Some histidine-tagged proteins are expressed as inclusion bodies in *E. coli*. Inclusion bodies are insoluble aggregates of misfolded proteins lacking biological activity. The problem can be solved by using a simple, but efficient combined purification and on-column refolding process to produce pure and active protein. A histidine-tagged scFv 57P antibody fragment expressed as inclusion bodies in *E. coli* was solubilized in guanidine hydrochloride and applied to a HisTrap HP column. Contaminants were removed followed by on-column refolding via buffer exchange to a nondenaturing buffer. A binding assay between a peptide derived from the tobacco mosaic virus and the eluate (refolded histidine-tagged protein) was performed using surface plasmon resonance (SPR) on a Biacore™ system.

Sample: 10 ml of solubilized histidine-tagged single chain Fv antibody fragment (Fab 57)

Column: HisTrap HP 1 ml

Solubilizing buffer: 20 mM Tris-HCl, 6 M Gua-HCl, 1 mM DTE, 1 mM Na_2-EDTA, 0.1 mM Pefabloc™, pH 7.5

Denatured binding 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 oxidized glutathione (GSSG), pH 7.5

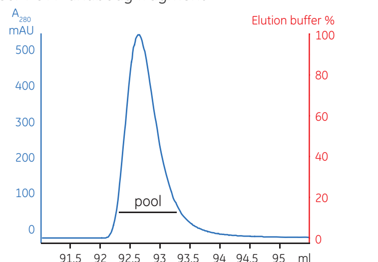
Native binding buffer: 20 mM Tris-HCl, 10 mM imidazole, 0.5 M NaCl, pH 7.5

Native elution buffer: 20 mM Tris-HCl, 500 mM imidazole, 0.5 M NaCl, pH 7.5

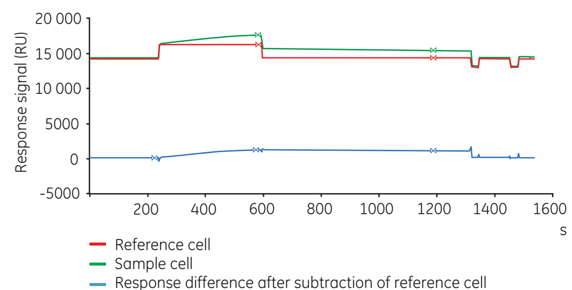
Flow rate: 1 ml/min

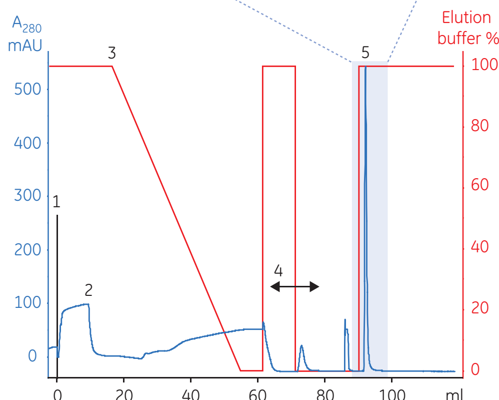
System: ÄKTAexplorer 10

Pooled fraction from the purification of refolded scFv 57P antibody fragment



SPR sensogram





1. Sample application
2. Wash
3. Start refolding
4. Equilibration of column with native binding buffer
5. Start elution

Summary

Using a combined purification and on-column refolding procedure produced 14% of active, refolded protein.

Unattended four-step purification and automatic tag removal

Products featured: HisTrap HP, HiPrep 26/10 Desalting, RESOURCE Q, HiLoad 16/60 Superdex 75 pg, ÄKTAxpress, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

ÄKTAxpress enables optimization of cleavage conditions of the histidine tag and automates the task of multistep purification. Optimization of AcTEV™ protease cleavage conditions for histidine-tagged APC1040 was determined using ÄKTAxpress. A four-step purification protocol including on-column tag cleavage using the optimized cleavage conditions obtained was then performed to achieve high amounts of pure APC1040.

Sample: APC1040-$(His)_6$ in *E. coli* lysate

Columns: AC: HisTrap HP 5 ml

DS: HiPrep™ 26/10 Desalting

IEX: RESOURCE™ Q, 6 ml

GF: HiLoad 16/60 Superdex 75 pg

Cleavage conditions: 200 units of AcTEV protease/mg protein, 8 h incubation time at room temperature

AC binding buffer: 50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.5

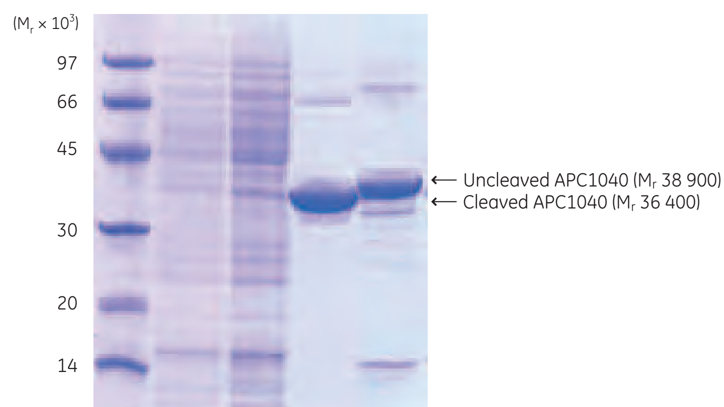
AC cleavage buffer: 50 mM Tris-HCl, 500 mM NaCl, 50 mM imidazole, pH 7.5

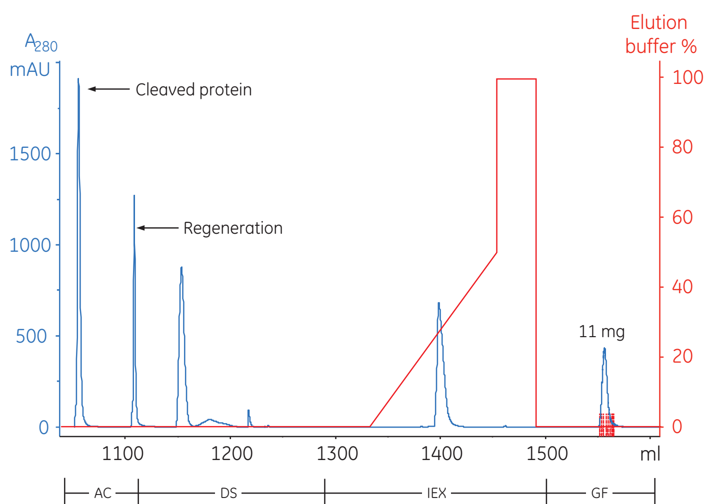
AC elution buffer: 50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.5

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





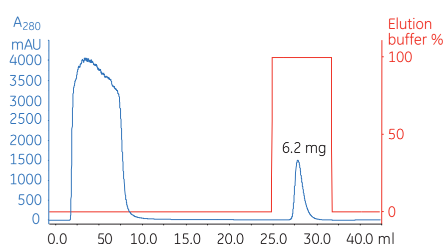
Summary
The efficient, four-step purification protocol effectively removed the histidine tag from the protein and yielded 11 mg of a highly pure APC1040.

Increasing the purification scale

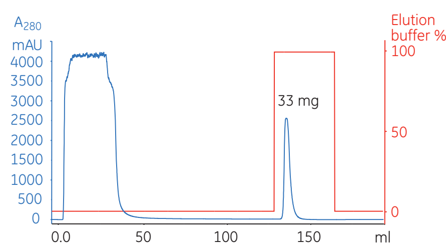
Products featured: HisTrap FF columns, HisPrep FF 16/10, ÄKTAexplorer, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

To obtain sufficient material for characterization studies, a scale-up experiment of a histidine-tagged maltose binding protein (MBP-[His]$_6$) was performed. The same protein load and linear flow rates were used on all three columns. Recovery and purity of the eluted material was determined and compared for all three runs.

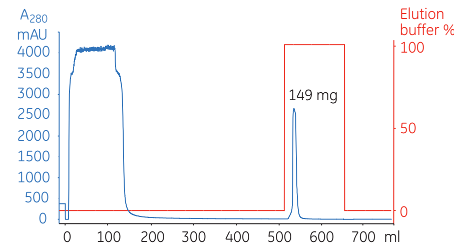
HisTrap FF 1 ml



HisTrap FF 5 ml



HisPrep FF 16/10



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

Sample: MBP-(His)$_6$, in *E. coli* extract

Sample volumes: 5.3 ml (1 ml column), 26.5 (5 ml column), 106 ml (20 ml column)

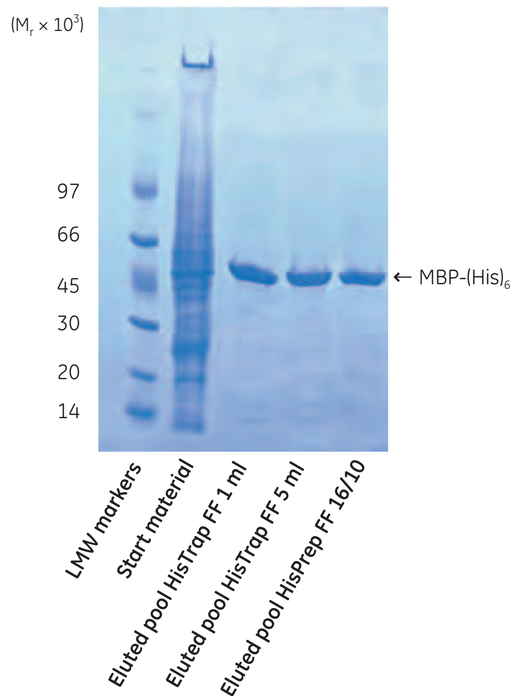
Binding buffer: 20 mM sodium phosphate, 25 mM imidazole, 500 mM NaCl, pH 7.4

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

System: ÄKTAexplorer 100

SDS-PAGE



Summary

The three different scale-up purifications produced similar results in terms of purity and recovery.

Ordering information

Product	Quantity	Code No.
Purification		
HisTrap HP	5 × 1 ml	17-5247-01
	100 × 1 ml†	17-5247-05
	1 × 5 ml	17-5248-01
	5 × 5 ml	17-5248-02
	100 × 5 ml†	17-5248-05
His MultiTrap HP	4 × 96-well filter plates	28-4009-89
His SpinTrap	50 × 100 µl	28-4013-53
His SpinTrap Kit	50 × 100 µl, buffers	28-9321-71
HisTrap FF	5 × 1 ml	17-5319-01
	100 × 1 ml†	17-5319-02
	5 × 5 ml	17-5255-01
	100 × 5 ml†	17-5255-02
HisTrap FF crude	5 × 1 ml	11-0004-58
	100 × 1 ml†	11-0004-59
	5 × 5 ml	17-5286-01
	100 × 5 ml†	17-5286-02
HisTrap FF crude Kit	3 × 1 ml, buffers	28-4014-77
HisPrep FF 16/10	1 × 20 ml	17-5256-01
His MultiTrap FF	4 × 96-well filter plates	28-4009-90
His GraviTrap	10 × 1 ml	11-0033-99
His GraviTrap Kit	20 × 1 ml, buffers	28-4013-51

† *available by specific customer order*

Product	Quantity	Code No.
Detection		
Anti-His antibody	170 µl	27-4710-01
ECL Mouse IgG HRP-linked Whole Ab	100 µl	NA931-100 µl
Amersham Hybond ECL (20 × 20 cm)	10 sheets	RPN2020D
Amersham Hyperfilm ECL (18 × 24 cm)	50 sheets	28-9068-36
Related products		
His Buffer Kit	1	11-0034-00
Ni Sepharose High Performance	25 ml	17-5268-01
	100 ml	17-5268-02
Ni Sepharose 6 Fast Flow	5 ml	17-5318-06
	25 ml	17-5318-01
	100 ml	17-5318-02
	500 ml	17-5318-03
HiLoad 16/60 Superdex 30 pg	1 × 120 ml	17-1139-01
HiLoad 26/60 Superdex 30 pg	1 × 320 ml	17-1140-01
HiLoad 16/60 Superdex 75 pg	1 × 120 ml	17-1068-01
HiLoad 26/60 Superdex 75 pg	1 × 320 ml	17-1070-01
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 200 pg	1 × 320 ml	17-1071-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
RESOURCE Q 6 ml	1	17-1179-01
ExcelGel SDS Gradient 8-18	6	80-1255-53
PhastGel™ Gradient 10-15	10	17-0540-01
LMW-SDS Marker Kit	10 vials	17-0446-01
Amersham High-Range Rainbow Molecular Weight Markers	250 µl	RPN756E

Strep-tag II proteins

Strep-tag II is a small tag of only eight amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) with a molecular weight of just 1000. The small size of the tag is very beneficial, since in most cases it does not interfere with structural and functional studies and, therefore, does not have to be removed.

Strep-tag II binds specifically to *Strep*-Tactin™ ligand immobilized on a Sepharose base matrix to yield pure target protein. The binding affinity of the *Strep*-tag II to the immobilized ligand is nearly 100-fold greater than to streptavidin, making StrepTactin Sepharose High Performance suitable for purifying *Strep*-tag II proteins. Purifications are run under physiological conditions, and mild elution with desthiobiotin preserves the activity of the target protein.

Simple two-step purification

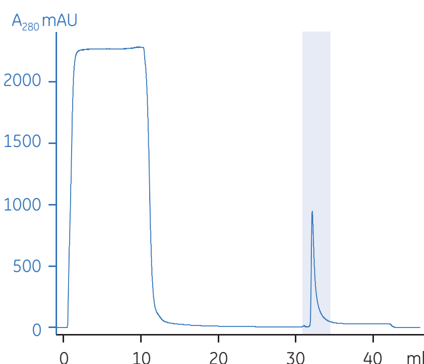
Products featured: StrepTrap HP, HiLoad 16/60 Superdex 75 pg, ÄKTAprime plus, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

Highly pure *Strep*-tag II proteins can be obtained with a simple chromatography system such as ÄKTAprime plus. $(His)_6$-mCherry-*Strep*-tag II (M_r ~31 000) was purified from an *E. coli* lysate using a two-step protocol involving affinity chromatography with a StrepTrap™ HP 1 ml column followed by gel filtration.

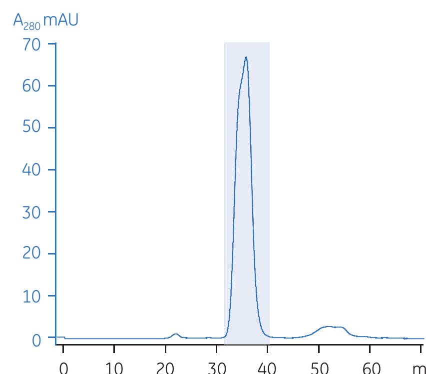
AC step:
Column: StrepTrap HP 1 ml
Sample: 10 ml of *E. coli* clarified lysate containing $(His)_6$-mCherry-*Strep*-tag II
Flow rate: 1 ml/min
Binding buffer: 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0
Elution buffer: 2.5 mM desthiobiotin in binding buffer

GF step:
Column: HiLoad 16/60 Superdex 75 pg
Sample: Eluted pool (2 ml) from StrepTrap HP 1 ml
Flow rate: 1 ml/min
Buffer: PBS buffer, pH 7.4
System: ÄKTAprime plus

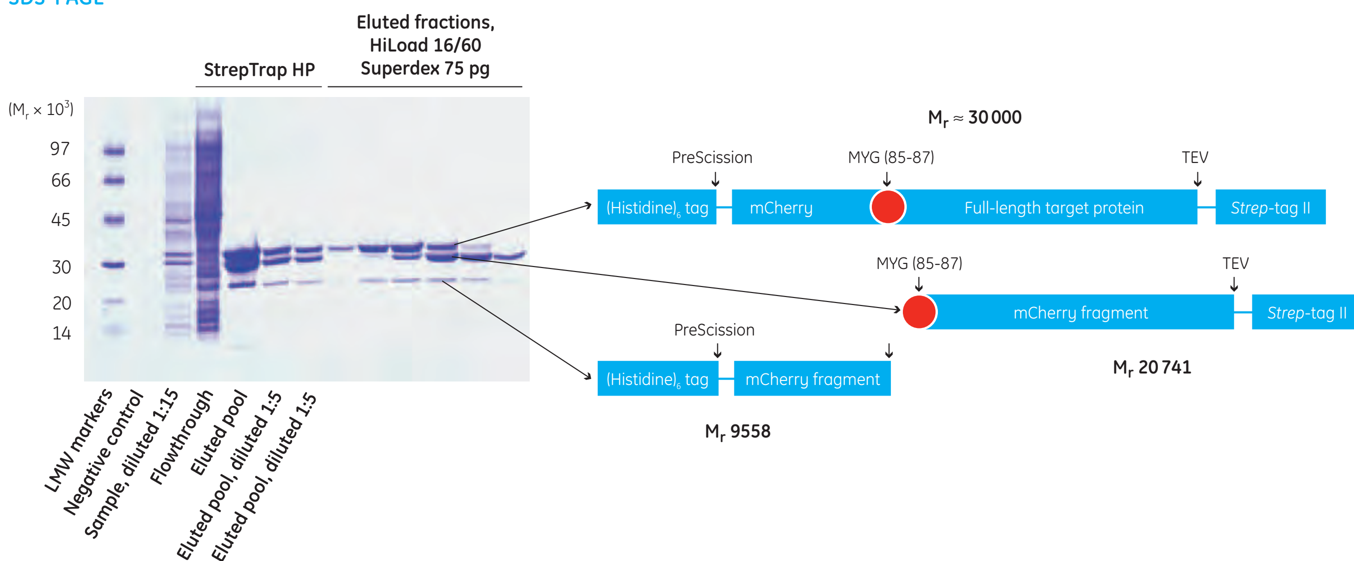
AC step



GF step



SDS-PAGE



Summary

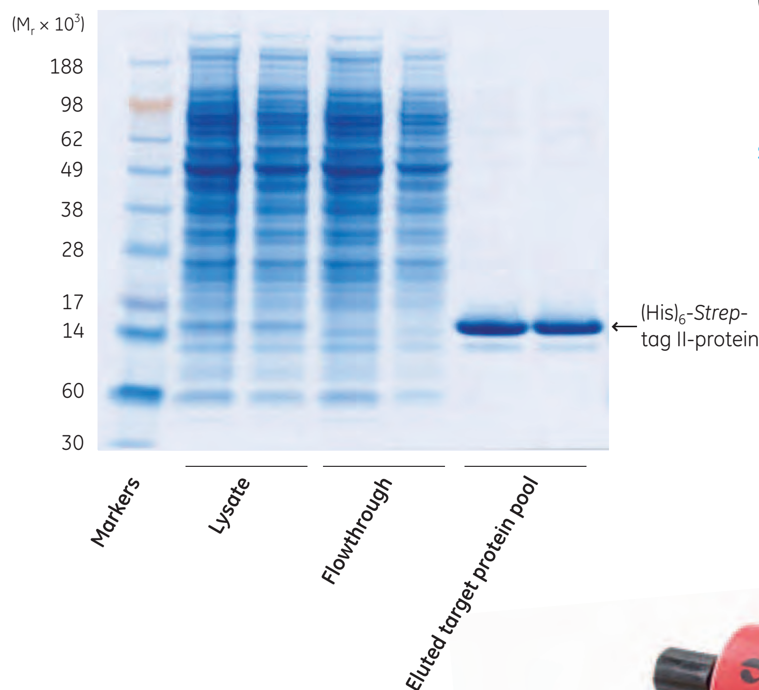
Highly pure *Strep*-tag II recombinant protein was obtained with this simple two-step purification protocol. The two contaminants of about M_r 10 000 and 21 000, respectively may be caused by fragmentation of the target protein during SDS-PAGE analysis.

Unattended purification of a protein expressed in insect cells

Products featured: StrepTrap HP, ÄKTAxpress

$(His)_6$-*Strep*-tag II-protein (M_r 15 400) was expressed in insect cells from a Baculovirus expression vector and purified. The high specificity of the *Strep*-tag II to the *Strep*-Tactin ligand was utilized in a one-step purification procedure using StrepTrap HP column to obtain highly pure target protein.

SDS-PAGE



Column: StrepTrap HP 5 ml

Sample: 175 ml of insect cell lysate containing $(His)_6$-*Strep*-tag II-protein (M_r ~15 400)

Binding buffer: 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0

Elution buffer: 2.5 mM desthiobiotin in binding buffer

Flow rates: 5 ml/min (1 ml/min during sample application)

System: ÄKTAxpress

Acknowledgements: M. Nilsson, R. Svensson and E. Holmgren, Biovitrum, Stockholm, Sweden

Summary

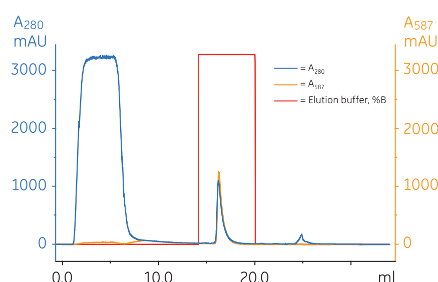
This simple, single-step process produced 3.7 mg of highly pure protein.

Increasing the purification scale

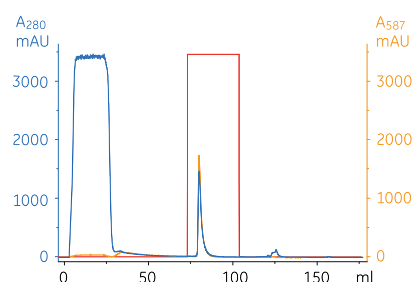
Products featured: StrepTrap HP columns, XK 26/20 column, StrepTactin Sepharose High Performance, ÄKTAexplorer

Scale-up of proteins can be achieved by increasing the bed volume while keeping the residence time constant. This approach maintains chromatographic performance during scale-up. The protein used was a fluorescent protein, $(His)_6$-mCherry-*Strep*-tag II, in *E. coli* lysate, which can be detected at 587 nm as well as 280 nm. Purification on a StrepTrap HP 1 ml column was first performed and then scaled up to the 5 ml column followed by further scale-up to a 29 ml XK 26/20 column packed with StrepTactin Sepharose High Performance. The protein load was increased five-fold in each scale-up step.

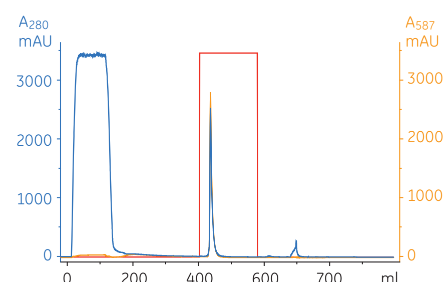
StrepTrap HP 1 ml



StrepTrap HP 5 ml



StrepTactin Sepharose High Performance XK 26/20



Columns: StrepTrap HP 1 ml, StrepTrap HP 5 ml, StrepTactin Sepharose High Performance packed in XK 26/20, 29 ml, bed height 5.5 cm

Sample: $(His)_6$-mCherry-*Strep*-tag II (M_r ~31 000), in *E. coli* lysate

Sample volumes: 4.2 ml (StrepTrap HP 1 ml), 21 ml (StrepTrap HP 5 ml), 105 ml (XK 26/20 column)

Regeneration: 3 column volumes (CV) distilled water, 3 CV 0.5 M NaOH, 3 CV distilled water

Binding buffer: 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0

Elution buffer: 2.5 mM desthiobiotin in binding buffer

Flow rates: StrepTrap HP 1 ml: 1.0 ml/min (0.5 ml/min during sample loading and regeneration with 0.5 M NaOH).

StrepTrap HP 5 ml: 5.0 ml/min (2.5 ml/min during sample loading and regeneration with 0.5 M NaOH).

XK 26/20 column: 13 ml/min (6.5 ml/min during regeneration with 0.5 M NaOH)

System: ÄKTAexplorer

Summary

The columns gave comparable results, confirming the ease and reproducibility of scaling up the purification from StrepTrap HP columns to a larger XK 26/20 column packed with StrepTactin Sepharose High Performance.

Ordering information

Product	Quantity	Code No.
Purification		
StrepTrap HP	5 × 1 ml	28-9075-46
	1 × 5 ml	28-9075-47
	5 × 5 ml	28-9075-48
StrepTactin Sepharose High Performance	10 ml	28-9355-99
	50 ml	28-9356-00
Related products		
HiLoad 16/60 Superdex 30 pg	1 × 120 ml	17-1139-01
HiLoad 26/60 Superdex 30 pg	1 × 320 ml	17-1140-01
HiLoad 16/60 Superdex 75 pg	1 × 120 ml	17-1068-01
HiLoad 26/60 Superdex 75 pg	1 × 320 ml	17-1070-01
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 200 pg	1 × 320 ml	17-1071-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
ExcelGel SDS Gradient 8-18	6	80-1255-53
LMW-SDS Marker Kit	10 vials	17-0446-01

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MBP-tagged proteins

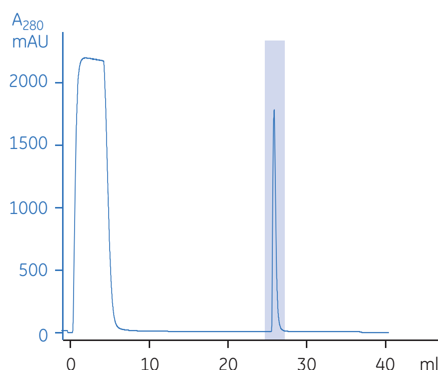
Maltose binding protein (MBP) is a useful affinity tag that can increase the expression level and solubility of the resulting tagged protein. The MBP tag also promotes proper folding of the attached protein. Since MBP increases solubility, the tag is particularly useful for recombinant proteins that accumulate in an insoluble form (inclusion bodies).

Affinity purification takes place under physiological conditions and mild elution is performed using maltose. The mild elution preserves the activity of the MBP-tagged protein.

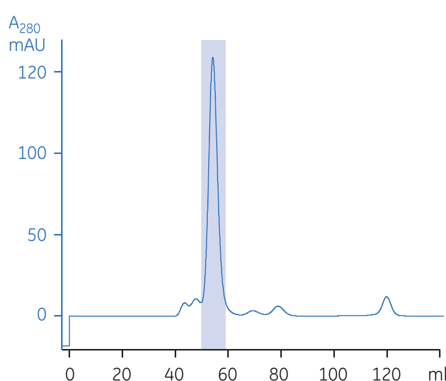
Simple two-step purification

Products featured: MBPTrap HP, HiLoad 16/60 Superdex 200 pg, ÄKTAprime plus, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

MBP*2-paramyosin-δ-Sal was purified in two steps with an MBPTrap™ HP 1 ml column, followed by gel filtration. The purification process was simplified with preprogrammed methods and optimized protocols on an ÄKTAprime plus.

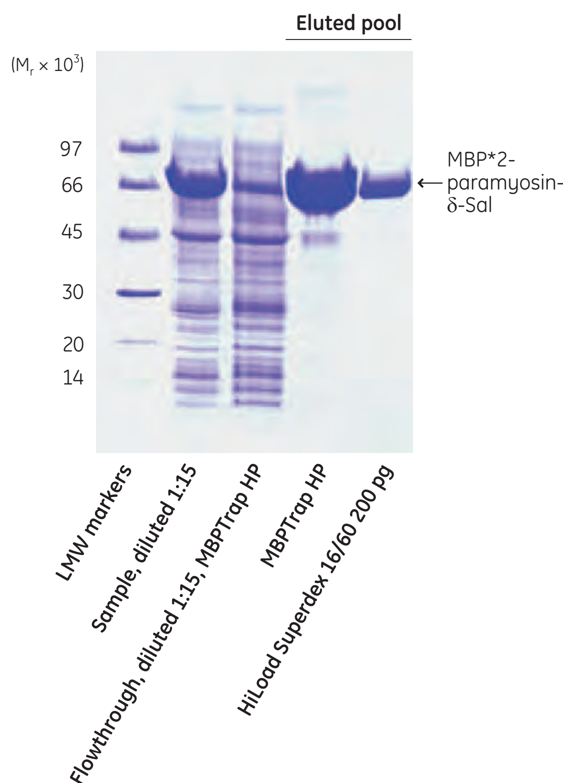


AC step:
Column: MBPTrap HP 1 ml
Sample: 4 ml MBP*2-paramyosin-δ-Sal in clarified *E. coli* lysate
Flow rate: 1 ml/min
Binding buffer: 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer: 10 mM maltose in binding buffer



GF step:
Column: HiLoad 16/60 Superdex 200 pg
Sample: Eluted pool (2 ml) from MBPTrap HP 1 ml
Flow rate: 1 ml/min
Buffer: PBS buffer, pH 7.4

SDS-PAGE

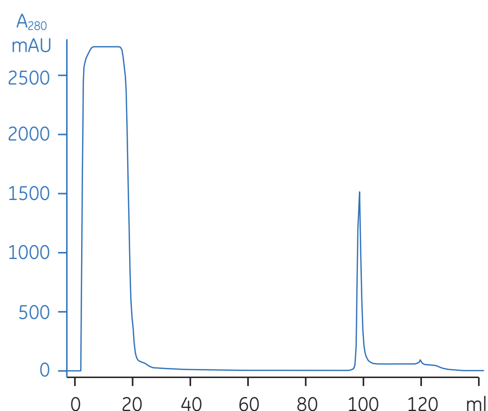


Summary
Inclusion of the second gel filtration step effectively removed impurities and contaminants to produce a pure target protein.

Efficient two-step purification of a protein involved in metabolic disease

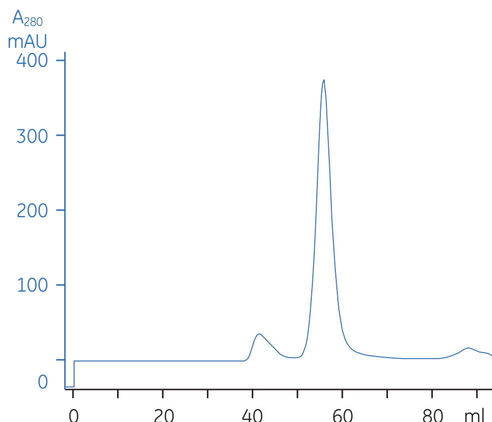
Products featured: MBPTrap HP, Superdex 200 prep grade, XK 16/20, ÄKTAprime

MCAD (M_r 85 500) is a homotetramer protein involved in metabolic disease. In this study, MCAD was purified for stability folding and kinetic studies. The purity of the eluted fractions was determined by SDS-PAGE analysis. Some truncated forms of the target protein, as well as protein aggregates were detected from the first step and were effectively removed in the second step.



AC step
Column: MBPTrap HP 5 ml
Sample: 15 ml of N-terminal MBP-MCAD in *E. coli* lysate
Binding buffer: 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer: 10 mM maltose in binding buffer
Flow rate: 5.0 ml/min (0.5 ml/min during sample loading)
System: ÄKTAprime

SDS-PAGE



GF step
Column: Superdex 200 pg in XK 16/20
Sample: 2 ml of eluted fraction from MBPTrap HP 5 ml
Buffer: 20 mM HEPES, 200 mM NaCl, pH 7.0
Flow rate: 0.4 ml/min
System: ÄKTAprime

Acknowledgements: E. M. Maier, Dr. von Haunersches Kinderspital, Munich, Germany

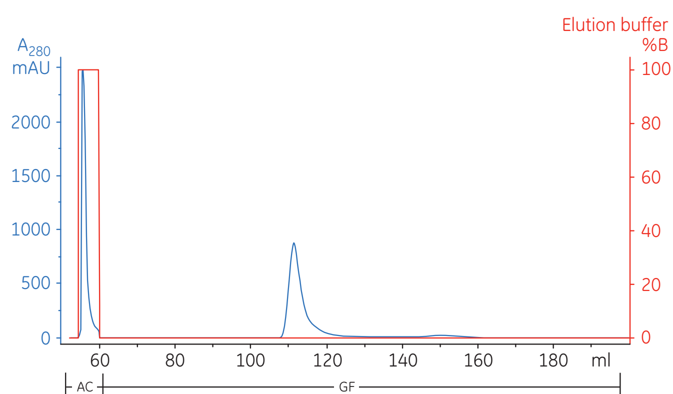
Summary

The target protein was highly concentrated and it eluted in a small volume from the first affinity step. This removes the need to concentrate the protein prior to gel filtration thus saving time, cost, and protein sample.

Unattended automated two-step purification

Products featured: MBPTrap HP, HiLoad 16/60 Superdex 200 pg, ÄKTAxpress

MBP-tagged apoptin protein (M_r ~ 60 000) was purified in a two-step procedure using ÄKTAxpress with affinity chromatography (AC) and gel filtration (GF) protocols. The protein was intended for crystallization screening and functional studies. The eluted peak from the MBPTrap column was automatically collected in a loop and injected onto the gel filtration column.



AC column: MBPTrap HP 5 ml

Sample: 15 ml of MBP-apoptin in *E. coli* lysate, M_r ~60 000

Flow rate: 5 ml/min

Binding buffer: 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4

Elution buffer: 10 mM maltose in binding buffer

GF column: HiLoad 16/60 Superdex 200 pg

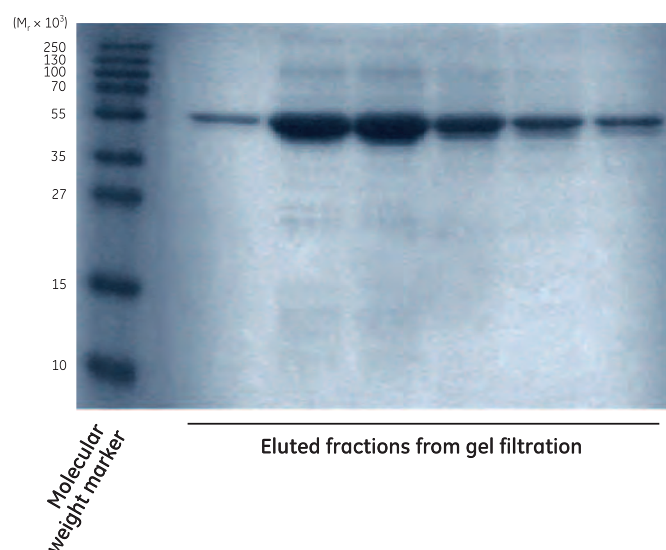
Sample: Collected pool from MBPTrap HP

Flow rate: 0.3 ml/min

Buffer: 10 mM sodium phosphate, 140 mM NaCl, 0.5 M EDTA, pH 7.2

System: ÄKTAxpress

SDS-PAGE



Acknowledgements: R. Zimmerman, Leiden University, 2333CC Leiden, The Netherlands

Summary

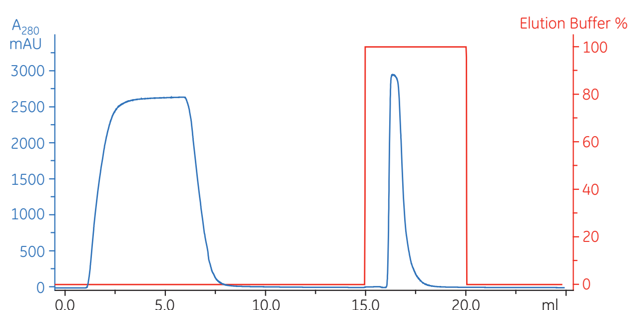
The use of only two steps in an automated protocol with ÄKTAxpress increases accuracy, allows for hands-off operation, and eliminates human error.

Increasing the purification scale

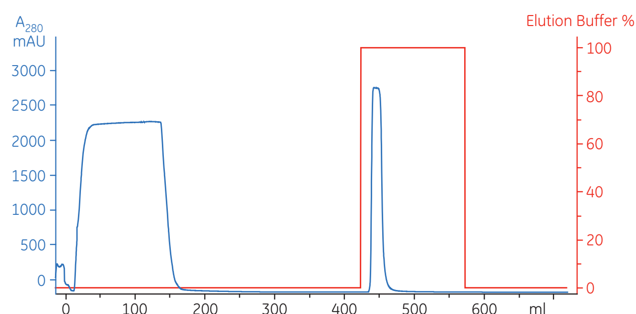
Products featured: MBPTrap HP columns, XK 26/20 column, Dextrin Sepharose High Performance, ÄKTAexplorer, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

To investigate the reproducibility of scale-up using MBPTrap columns, MBP2*-β-galactosidase (M_r ~158 000), a recombinant tagged multimer, was purified on MBPTrap HP 1 ml and 5 ml columns, which are prepacked with Dextrin Sepharose High Performance medium. Further scale-up was performed on an XK 26/20 column, packed with the same chromatography medium. Sample load was increased five-fold for each scale-up step.

MBPTrap HP 1 ml



Dextrin Sepharose High Performance XK 26/20



Columns: MBPTrap HP 1 ml, MBPTrap HP 5 ml, Dextrin Sepharose High Performance packed in XK 26/20, 29 ml, bed height 5.5 cm

Sample: MBP2*-β-galactosidase (M_r ~158 000) in *E. coli* lysate

Sample volumes: 5 ml (MBPTrap HP 1 ml), 25 ml (MBPTrap HP 5 ml), 125 ml (XK 26/20 column)

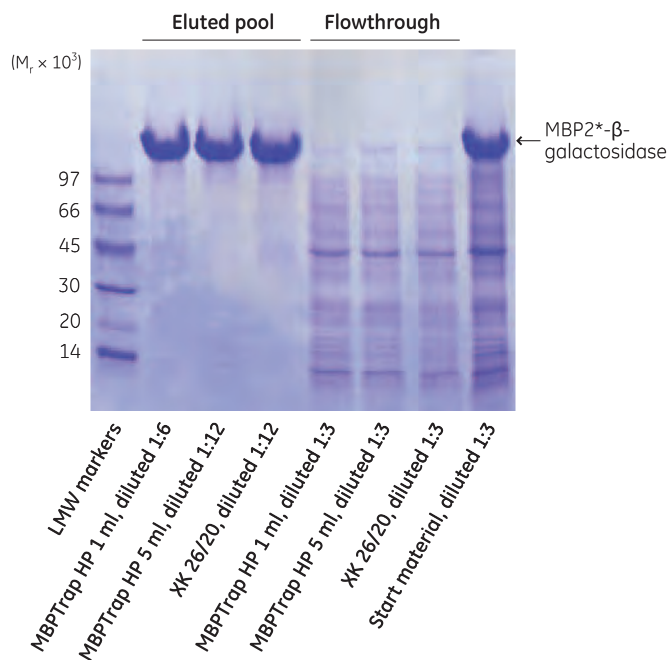
Binding buffer: 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4

Elution buffer: 10 mM maltose in binding buffer

Flow rates: MBPTrap HP 1 ml: 1.0 ml/min (0.5 ml/min during sample loading)
MBPTrap HP 5 ml: 5.0 ml/min (2.5 ml/min during sample loading)
XK 26/20 column: 13 ml/min

System: ÄKTAexplorer

SDS-PAGE



Summary

The columns gave comparable results with high purity and similar yields (about 60%, data not shown), confirming the ease and reproducibility of scaling up purifications from MBPTrap HP columns to an XK 26/20 column packed with the same chromatography medium.

Ordering information

Product	Quantity	Code No.
Purification		
MBPTrap HP	5 × 1 ml	28-9187-78
	1 × 5 ml	28-9187-79
	5 × 5 ml	28-9187-80
Dextrin Sepharose High Performance	25 ml	28-9355-97
	100 ml	28-9355-98
Purification		
HiLoad 16/60 Superdex 30 pg	1 × 120 ml	17-1139-01
HiLoad 26/60 Superdex 30 pg	1 × 320 ml	17-1140-01
HiLoad 16/60 Superdex 75 pg	1 × 120 ml	17-1068-01
HiLoad 26/60 Superdex 75 pg	1 × 320 ml	17-1070-01
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 200 pg	1 × 320 ml	17-1071-01
ExcelGel SDS Gradient 8-18	6	80-1255-53
LMW-SDS Marker Kit	10 vials	17-0446-01
Superdex 200 prep grade	150 ml	17-1043-01
XK 16/20 empty column	1	18-8773-01

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Dual-tagged proteins

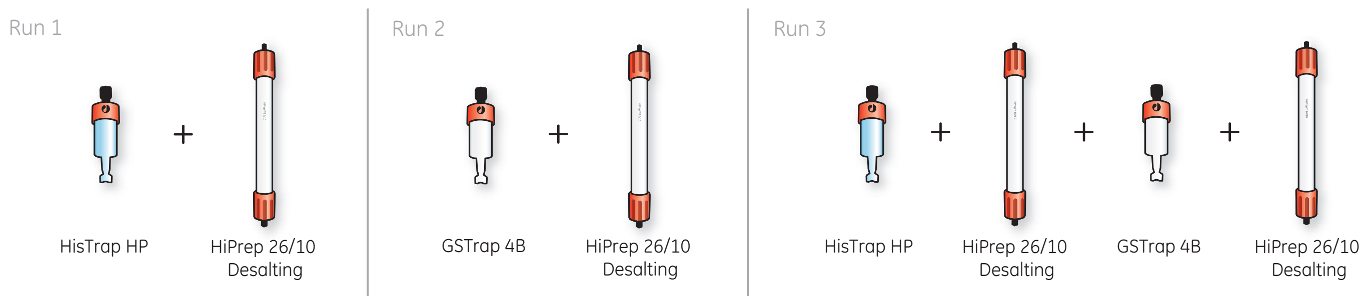
Recombinant proteins can be designed to contain N- or C-terminal affinity tags. The inclusion of two different tags in a construct results in a target protein that can be purified with different affinity chromatography media thus producing a purer protein with a straightforward purification scheme.

If different tags are positioned on each end of the protein, a dual-tagged purification scheme would normally produce a full-length protein. The affinity tag can be cleaved if cleavage sites are included between the tag and the target protein.

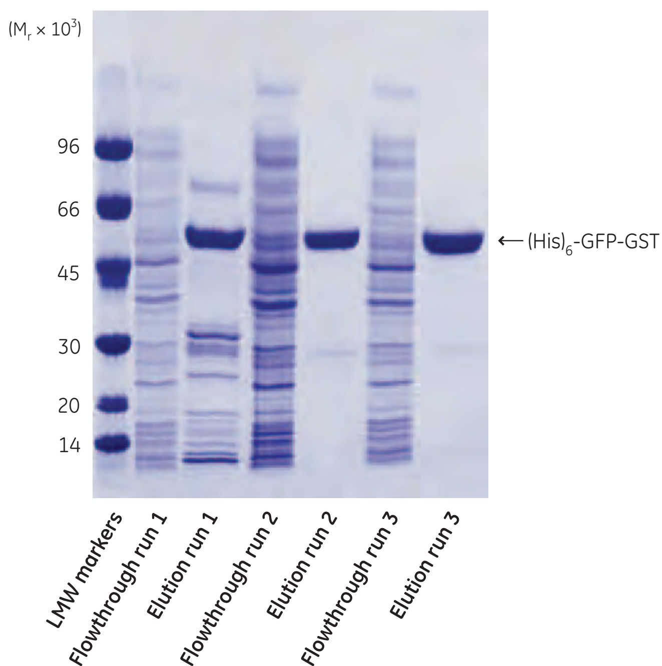
One versus two affinity steps

Products featured: HisTrap HP, GSTrap 4B, ÄKTAxpress, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

To compare purification using either one tag or a combination of two different tags, a dual-tagged green fluorescent protein ($[His]_6$-GFP-GST) was purified in three different ways. Purification on HisTrap HP showed that the medium had affinity for other native proteins with exposed histidine residues as well, whereas utilizing the high specificity of the GST tag for the glutathione ligand on GSTrap 4B gave a much purer protein. The combination of the two tags produced the highest purity.



SDS-PAGE



Sample: $(His)_6$-GFP-GST in *E.coli* lysate

Columns:

Affinity chromatography: HisTrap HP 5 ml and GSTrap 4B 5 ml

Desalting: HiPrep 26/10 Desalting

Buffers:

HisTrap HP

Binding buffer: 20 mM PBS, 20 mM imidazol, 0.5 M NaCl, pH 7.4

Elution buffer: 20 mM PBS, 500 mM imidazol, 0.5 M NaCl, pH 7.4

GSTrap 4B:

Binding buffer: 10 mM PBS, pH 7.4

Elution buffer: 50 mM Tris, 20 mM reduced glutathone, pH 8

HiPrep 26/10 Desalting:

Buffer: 50 mM Tris-HCl , 150 mM NaCl, pH 7.5

Summary

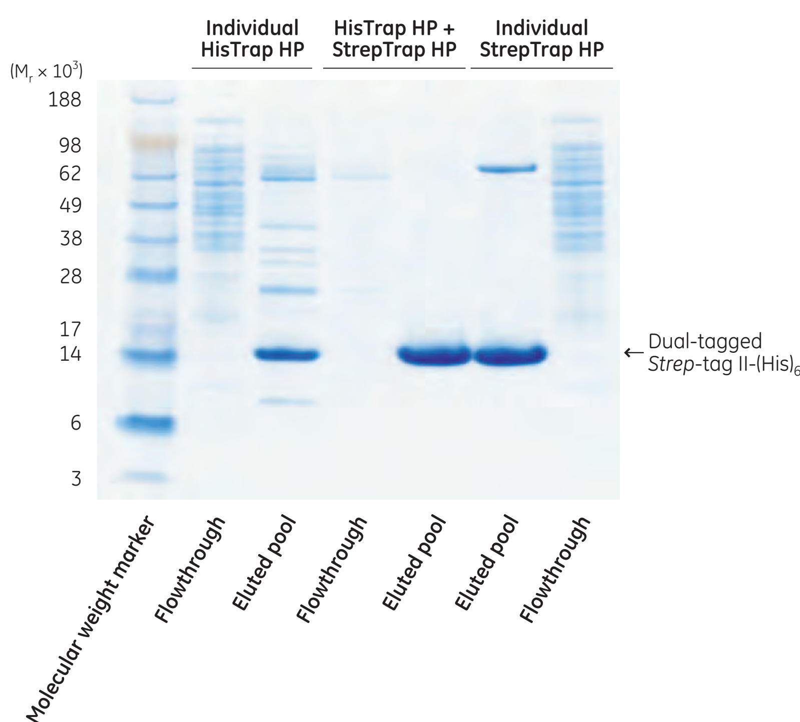
Dual-tagged purification confers a synergistic benefit (higher purity) that sometimes eludes single-tagged purification schemes.

Increased purity with dual-tagged expressed protein

Products featured: HisTrap HP, StrepTrap HP, ÄKTAxpress

Since a high degree of purity is crucial for successful functional studies, purity results of the two-step method were compared with those from single-step purifications. A dual-tagged *Strep* tag II-(histidine)$_6$ protein (M_r ~15 400) expressed in *E. coli* was purified for method development using a two-step procedure comprising immobilized metal affinity chromatography (IMAC) followed by affinity chromatography on a StrepTrap HP column. All the experiments were conducted at 4°C to preserve protein stability.

SDS-PAGE



Sample: 15 ml *Strep*-tag II-(histidine)$_6$ protein (M_r ~15 400) in *E. coli* lysate

Individual HisTrap HP purification

Column: HisTrap HP 1 ml

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.5

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.5

Flow rate: 0.8 ml/min

Individual StrepTrap HP purification

Column: StrepTrap HP 1 ml

Binding buffer: 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0

Elution buffer: 2.5 mM desthiobiotin in binding buffer

Flow rate: 0.8 ml/min

Two-step HisTrap HP and StrepTrap HP purification

Column: HisTrap HP 1 ml

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.5

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.5

Flow rate: 0.8 ml/min

Column: StrepTrap HP 1 ml

Sample: Eluted fraction from HisTrap HP, 1 ml

Binding buffer: 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0

Elution buffer: 2.5 mM desthiobiotin in binding buffer

Flow rate: 0.2 ml/min

System: ÄKTAxpress

Acknowledgements: M. Nilsson, R. Svensson and E. Holmgren, Biovitrum, Stockholm, Sweden

Summary

SDS-PAGE results clearly demonstrate the benefits of a dual-tagged approach to protein purification, especially when high purity is required.

Impact of reversing the order of affinity purification

Products featured: StrepTrap HP, HisTrap HP, ÄKTAxpress, ExcelGel SDS Gradient 8–18, Multiphor II Electrophoresis System, LMW-SDS Marker Kit

To evaluate whether the order of affinity columns in a chosen purification method matters with respect to yield and purity. $(His)_6$-mCherry-*Strep*-tag II dual-tagged target protein, was purified using StrepTrap HP followed by HisTrap HP and subsequently in the reverse order. SDS-PAGE analysis showed that the purity of the protein was the same, irrespective of the order of affinity purification used. An N-terminally truncated target protein identified by mass spectrometry (data not shown), however, passed through the HisTrap HP column more slowly than nontagged proteins, which accounts for the two peaks observed in the flowthrough.

Columns: StrepTrap HP 1 ml, HisTrap HP 1 ml

Sample: $(His)_6$-mCherry-*Strep*-tag II in *E. coli* lysate

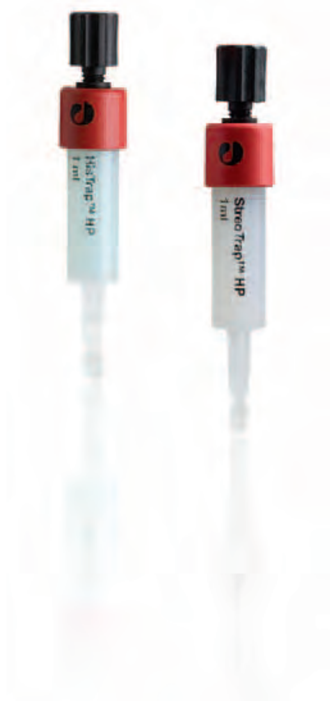
Binding buffer (StrepTrap HP): 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0

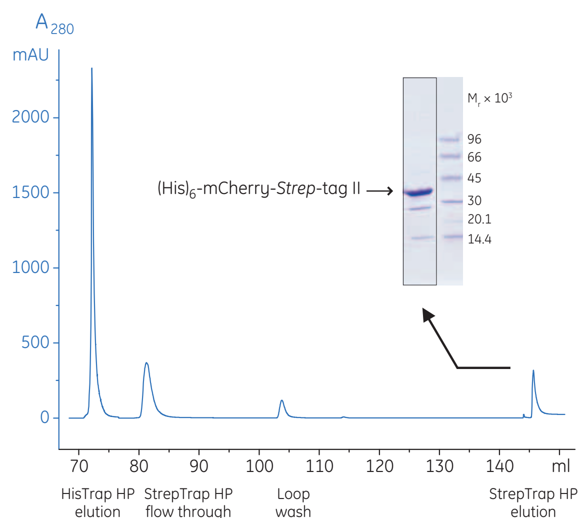
Elution buffer (StrepTrap HP): 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8.0

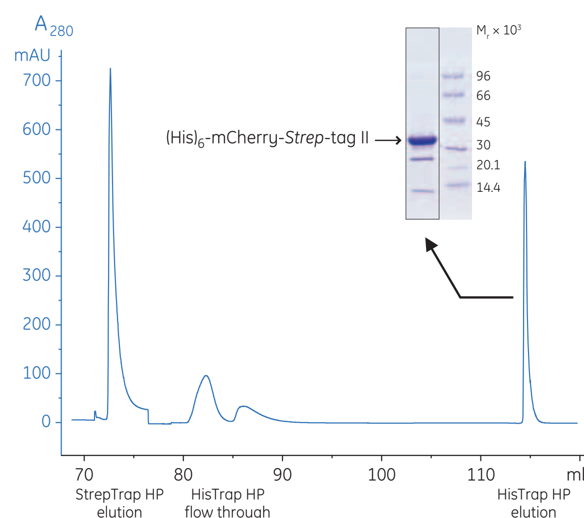
Binding buffer (HisTrap HP): 20 mM phosphate, 500 mM NaCl, 5 mM Imidazole, pH 7.4

Elution buffer (HisTrap HP): 20 mM phosphate, 500 mM NaCl, 500 mM Imidazole, pH 7.4

System: ÄKTAxpress







Summary

The same amount (0.9 mg) of protein was produced in both experiments and the purity of the target protein was not affected by the order of the affinity columns used.

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