StrepTactin **Sepharose** High Performance **StrepTrap** HP

AFFINITY PURIFICATION

StrepTactin™ Sepharose™ High Performance is a chromatography medium for purifying Strep(II)-tagged recombinant proteins. The medium is available in 10 ml and 50 ml lab packs and prepacked in 1 ml and 5 ml StrepTrap™ HP columns.

The Strep(II) tag is a small tag consisting of only eight amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and a relative molecular mass (M_r) of only 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 from the target protein. The Strep(II) tag binds very specifically to the immobilized StrepTactin ligand giving pure target protein. Affinity purification using StrepTactin Sepharose High Performance takes place under physiological conditions, and mild elution with desthiobiotin preserves the activity of the target protein.

StrepTactin Sepharose High Performance benefits:

- Highly pure Strep(II)-tagged recombinant proteins eluted in concentrated form and small volumes
- Physiological conditions and mild elution preserve target protein activity
- Fast and easy regeneration with 0.5 M NaOH
- Compatible with a wide range of reducing agents, detergents, denaturants and other additives
- Prepacked StrepTrap HP 1 ml and 5 ml columns offer convenience, save time, and ensure reproducible results
- Easy scale-up

Fig 1. StrepTactin Sepharose High Performance, also prepacked as StrepTrap HP columns, give fast and convenient affinity purifications of Strep(II)-tagged recombinant proteins.

Description

Chromatography medium characteristics

StrepTactin is a specially-engineered streptavidin ligand. The binding affinity of the Strep(II)-tag to the immobilized ligand is nearly 100-fold higher than to streptavidin, making StrepTactin Sepharose High Performance ideal for purifying Strep(II)-tagged proteins.

The small bead size (average 34 µm) of the Sepharose High Performance matrix results in high-resolution separations, sharp peaks and purified target proteins in a concentrated form. StrepTactin Sepharose High Performance is compatible with a wide range of additives, tolerates all commonly used aqueous buffers, and is quickly and easily regenerated using 0.5 M NaOH.

StrepTrap HP column characteristics

The columns are 1 ml and 5 ml HiTrap™ columns made of biocompatible polypropylene that does not interact with biomolecules. Prepacked StrepTrap HP columns, which provide fast and simple purifications in a convenient format, are delivered with a stopper on the inlet and a snap-off end on the outlet. Porous top and bottom frits allow high flow rates.

The columns can be operated either with a syringe, a peristaltic pump, an ÄKTA™ system, or other chromatography systems. Note that HiTrap columns cannot be opened or refilled.

Tables 1 and 2 summarize the characteristics of StrepTactin Sepharose High Performance and StrepTrap HP columns, respectively. Table 3 lists the compatibility of the StrepTactin Sepharose High Performance medium with different additives.

Table 1. Characteristics of StrepTactin Sepharose High Performance

Matrix	Rigid, highly cross-linked 6% agarose
Average particle size	34 µm
Ligand	StrepTactin
Ligand concentration	Approx. 5 mg/ml medium
Dynamic binding capacity[1]	Approx. 6 mg Strep(II)-tagged protein/ml medium
Recommended flow rate[2]	≤ 150 cm/h
Maximum linear flow rate[2]	< 300 cm/h
Chemical stability	Stable in all commonly used buffers, reducing agents, and detergents (see Table 3), as well as 0.5 M NaOH (regeneration and cleaning)
pH stability (working range)[3]	> 7
Storage	4°C to 8°C in 20% ethanol

[1] Binding capacity is protein dependent. Dynamic binding capacity was tested with GAPDH-Strep(II), M_r 37 400
[2] H_2O at room temperature
[3] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

Table 2. Characteristics of StrepTrap HP

Column volumes	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rates[1]	1 and 5 ml/min for 1 and 5 ml columns, respectively
Maximum flow rates[1]	4 and 20 ml/min for 1 and 5 ml columns, respectively
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)

[1] H_2O at room temperature

Table 3. Compatibility of StrepTactin Sepharose High Performance with different additives[1]

Reagent	Concentration
Reduction agents	
DTT	50 mM
β-mercaptoethanol	50 mM
Nonionic detergents	
C8E4, Octyltetraoxyethylene	max. 0.88%
C10E5, Decylpentaoxyethylene	0.12%
C10E6	0.03%
C12E8	0.005%
C12E9, Dodecyl nonaoxyethylene (Thesit)	0.023%
Decyl-β-D-maltoside	0.35%
N-dodecyl-β-D-maltoside	0.007%
N-nonyl-β-D-glucopyranoside	0.2%
N-octyl-β-D-glucopyranoside	2.34%
Triton™ X-100	2%
Tween™ 20	2%
Ionic detergents	
N-lauryl-sarcosine	2%
8-HESO; N-octyl-2-hydroxy-ethylsulfoxide	1.32%
SDS, Sodium-N-dodecyl sulfate	0.1%
Zwitterionic detergents	
CHAPS	0.1%
DDAO, N-decyl-N,N-dimethylamine-N-oxide	0.034%
LDAO, N-dodecyl-N,N-dimethylamine-N-oxide	0.13%
Others	
Ammonium sulfate, $(NH_4)_2SO_4$	2 M
$CaCl_2$	max. 1 M
EDTA	50 mM
Guanidine	max. 1 M
Glycerol	max. 25%[2]
Imidazole	500 mM[3]
$MgCl_2$	1 M
Urea	max. 1 M
NaCl	5 M

[1] Data kindly provided by IBA GmbH, Germany, the manufacturer and IP owner of the StrepTactin ligand
[2] Yield may decrease
[3] 500 mM imidazole in sample tested by Cytiva
Note: These reagents have been successfully tested for the purification of, for example, GAPDH-Strep(II) with concentrations up to those listed. Higher concentrations may, however, be possible for reagents not marked with "max". Since binding depends on the sterical accessibility of the Strep(II)-tag in the context of the particular protein, the possible concentration can deviate from the given value for other proteins

Use and applications

Packing in laboratory columns

StrepTactin Sepharose High Performance is supplied pre-swollen in 10 ml and 50 ml packs. The medium is easy to pack and use in, for example, laboratory columns from the Tricorn™ and XK series (see *Ordering information*). Full user instructions are supplied with each pack.

StrepTrap HP

Purifications on StrepTrap HP 1 ml and 5 ml are easily performed using a syringe and the provided Luer adapter (Fig 2), a laboratory pump, or a chromatography system such as ÄKTA systems.

(A)

(B)

(C)

Fig 2. Using StrepTrap HP 1 ml with a syringe. (A) Prepare buffers and sample. Remove the column's top cap and snap off the end. Wash and equilibrate. (B) Load the sample and begin collecting fractions. (C) Elute and continue collecting fractions.

Increased purity with a two-step affinity purification of a dual-tagged protein

A dual-tagged Strep(II)-(histidine)$_6$ protein (M_r ~15 400) expressed in *E. coli* was purified for method development of functional studies. The two-step procedure comprised immobilized metal affinity chromatography (IMAC) on HisTrap™ HP (prepacked with Ni Sepharose High Performance) followed by affinity chromatography on StrepTrap HP. As high purity is crucial for successful functional studies, purity results of the two-step method were compared to the IMAC and affinity chromatography steps individually. All runs were performed on ÄKTAxpress at 4°C. The conditions used are shown below:

Individual HisTrap HP purification

Column:	HisTrap HP 1 ml
Sample:	Strep(II)-(histidine)$_6$ protein (M_r ~15 400) in *E. coli* lysate
Sample volume:	15 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
System:	ÄKTAxpress

Individual StrepTrap HP purification

Column:	StrepTrap HP 1 ml
Sample:	Strep(II)-(histidine)$_6$ protein (M_r ~15 400) in *E. coli* lysate
Sample volume:	15 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
System:	ÄKTAxpress

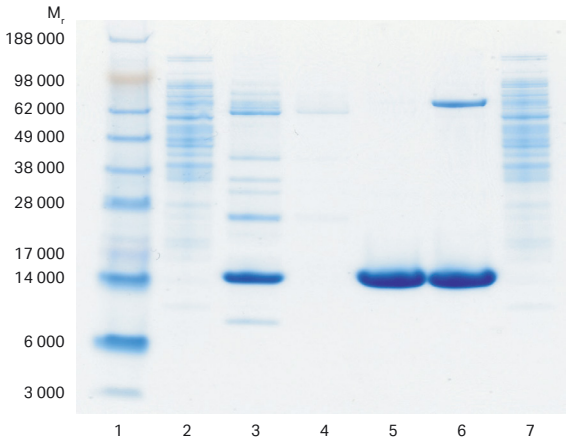
Two-step HisTrap HP and StrepTrap HP purification

Column:	HisTrap HP 1 ml
Sample:	Strep(II)-(histidine)$_6$ protein (M_r ~15 400) in *E. coli* lysate
Sample volume:	15 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
System:	ÄKTAxpress

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

SDS-PAGE analysis (Fig 3) showed that the individual HisTrap HP purification yielded the target protein and a number of different impurities (lane 3). StrepTrap HP on its own also yielded the target protein, this time with one impurity (lane 6). In contrast, the combination of HisTrap HP followed by StrepTrap HP resulted in a target protein with a purity greater than 95% (lane 5).

This example clearly demonstrates the benefits of a dual-tagged approach to protein purification, especially when high purity is needed for applications such as functional studies. HisTrap HP and StrepTrap HP run in sequence on ÄKTAxpress fulfilled the requirements for a fast and efficient chromatography system capable of delivering such results.



Lane
1. Molecular weight markers
2. Flowthrough, individual HisTrap HP
3. Eluted pool, individual HisTrap HP
4. Flowthrough, HisTrap HP + StrepTrap HP
5. Eluted pool, HisTrap HP + StrepTrap HP
6. Eluted pool, individual StrepTrap HP
7. Flowthrough, individual StrepTrap HP

Fig 3. SDS-PAGE analysis (reduced conditions) comparing individual purifications on HisTrap HP 1 ml and StrepTrap HP 1 ml with a combined, two-step affinity purification on both columns.

Regeneration with NaOH

Regenerating StrepTrap HP prior to performing the next purification will help retain chromatographic performance and promote cost-effective use. The columns can easily be regenerated using two protocols either with 0.5 M NaOH (approx. 10 min) or 1 mM HABA (2-[4′-hydroxy-benzeneazo] benzoic acid (approx. 25 min). This example shows regeneration with NaOH, which is fast, simple and effective. GAPDH-Strep(II) (M_r ~37 400) was purified on StrepTrap HP 1 ml. Six repetitive runs were made with regeneration using 0.5 M NaOH between each run. Figure 4 shows the overlayed chromatograms from these six runs, illustrating the very high reproducibility of StrepTrap HP columns. Further analysis by capillary electrophoresis demonstrated high purities (more than 97% in all six runs, data not shown), a result confirmed by SDS-PAGE analysis (Fig 5). Recovery was also high (greater than 80%) and very consistent throughout the entire study (Fig 6). The UV measurements were performed using a plate reader.

Column:	StrepTrap HP 1 ml
Sample:	Strep(II)-tagged glyceraldehyde-phosphodehydrogenase (GAPDH-Strep[II], M_r ~37 400), 1.0 mg/ml in *E. coli* lysate
Sample volume:	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
Regeneration:	3 ml distilled water, 3 ml 0.5 M NaOH, 3 ml distilled water
Re-equilibration:	5 ml binding buffer
Flow rate:	1.0 ml/min (0.5 ml/min with NaOH)
System:	ÄKTAexplorer

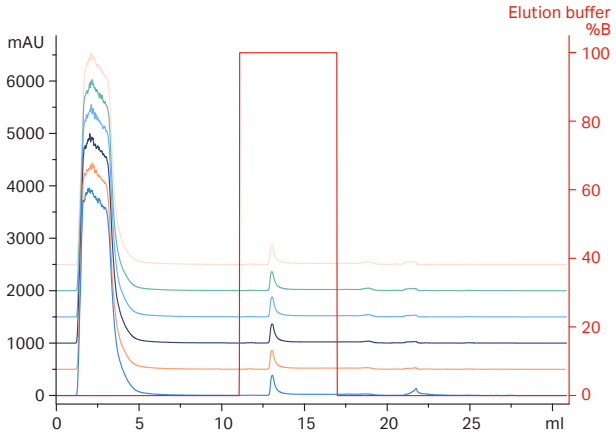
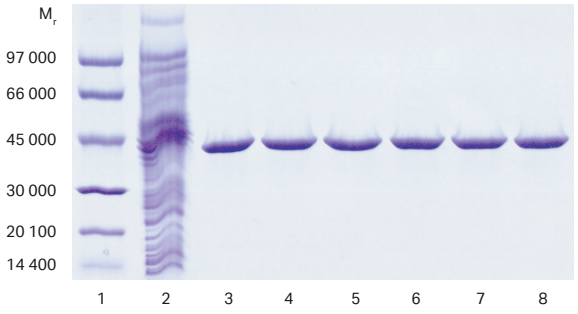


Fig 4. Six repetitive GAPDH-Strep(II) purification runs on the same StrepTrap HP 1 ml column.



Lane
1. Low molecular weight markers
2. Start material, *E. coli* lysate with GAPDH-Strep(II)
3. Run 1, eluted pool
4. Run 2, eluted pool
5. Run 3, eluted pool
6. Run 4, eluted pool
7. Run 5, eluted pool
8. Run 6, eluted pool

Fig 5. SDS-PAGE analysis (reduced conditions) of the six repetitive purifications runs in the regeneration study shows excellent reproducibility.

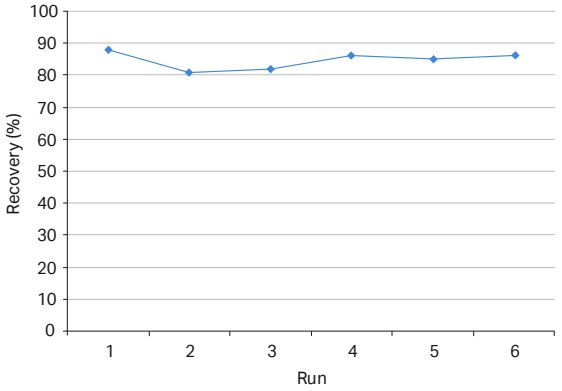


Fig 6. Recovery in the eluted fractions was retained over six purification runs.

Scaling up

Scale-up can be achieved by increasing the bed volume while keeping the residence time constant. This approach maintains chromatographic performance during scale-up. For quick scale-up, two or more StrepTrap HP columns can be connected in series by connecting columns in series. Note, however, that this may increase backpressure on the column. The scale-up study below describes the first approach.

The protein used was a dual-tagged fluorescent protein, $(His)_6$-mCherry-Strep(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 step and the residence time was ~2 min for all columns.

Figure 7 shows the chromatograms and running conditions. Protein load was increased five-fold for the scale-up from the 1 ml StrepTrap HP column to the 5 ml column and 25-fold in the scale-up from the 5 ml StrepTrap HP column to the 29 ml XK 26/20 column. Yield, calculated from absorbance measurements, was 2.2, 9.4 and 52.7 mg, respectively (Table 4). SDS-PAGE (data not shown) showed that the purity of the fractions eluted from the columns was similar.

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

Table 4. Overview of the yield for StrepTrap HP and XK 26/20 column.

Column	Yield (mg)
StrepTrap HP, 1 ml	2.2
StrepTrap HP, 5 ml	9.4
XK 26/20 packed with StrepTactin Sepharose High Performance, 29 ml	52.7

Column:	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(II) (M_r ~31 000), in *E. coli* lysate
Sample volume:	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 rate:	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

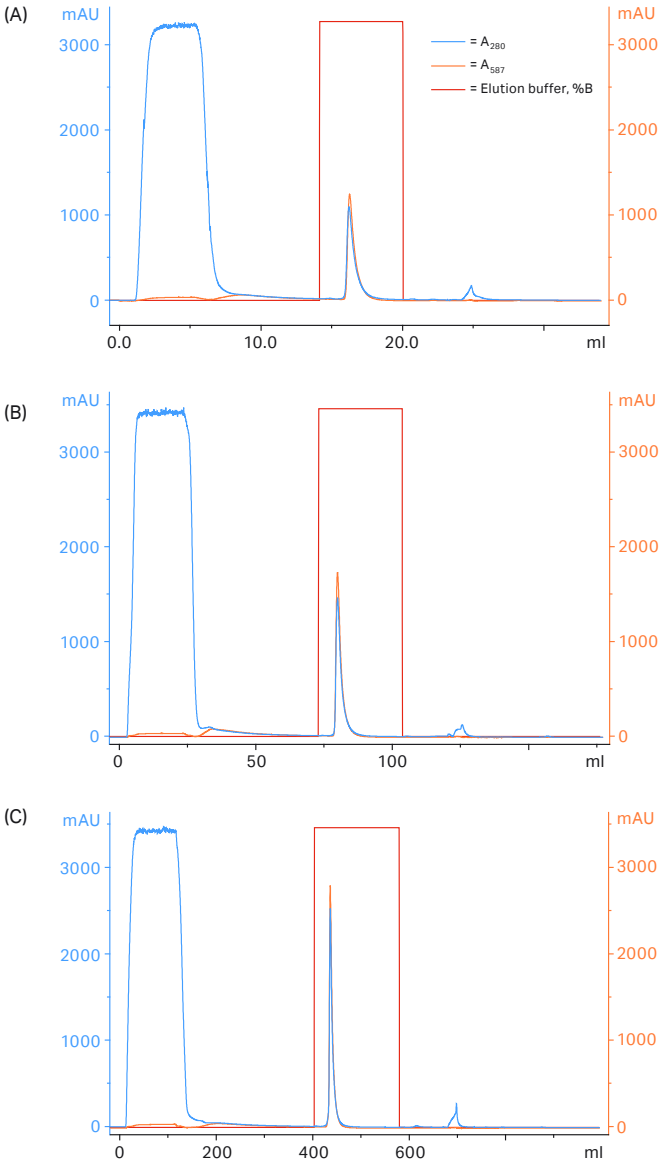


Fig 7. Scaling up the purification of $(His)_6$-mCherry-Strep(II), (A) StrepTrap HP 1 ml, (B) StrepTrap HP 5 ml, (C) StrepTactin Sepharose High Performance XK 26/20, 29 ml.

Reproducible, automated two-step purifications by affinity chromatography of $(His)_6$-mCherry-Strep(II)

The dual-tagged red fluorescent protein, $(His)_6$-mCherry-Strep(II) (M_r ~31 000), was purified using an automated two-step affinity chromatography purification on a StrepTrap HP 1 ml column (to bind the Strep[II] tag) and HisTrap HP 1 ml column (to bind the $[histidine]_6$ tag). The purification was run in automatic mode using the AC-AC protocol of ÄKTAxpress. To investigate reproducibility, three separate two-step purifications were performed.

The sample was first applied to three different 1 ml StrepTrap HP columns to bind the target protein and wash away the *E. coli* proteins, thereby reducing the risk for proteolytic degradation. The tagged protein was then sequentially eluted from the three StrepTrap HP columns and applied to a single HisTrap HP 1 ml column, where the three second-step affinity purifications were performed.

Column (AC 1):	StrepTrap HP 1 ml (× 3)
Sample:	$(His)_6$-mCherry-Strep(II) (M_r ~31 000), in *E. coli* lysate
Sample volume:	15 ml per column
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:	1.0 ml/min
System:	ÄKTAxpress
Column (AC 2):	HisTrap HP 1 ml
Sample:	Eluted pools from three different runs on StrepTrap HP, 1 ml
Binding buffer:	20 mM phosphate, 500 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	500 mM imidazole in binding buffer
Flow rate:	1.0 ml/min
System:	ÄKTAxpress

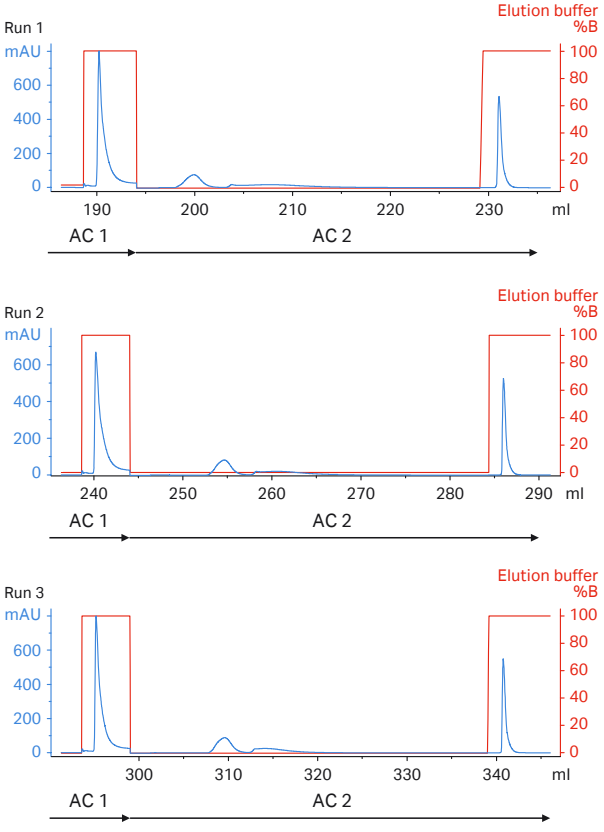
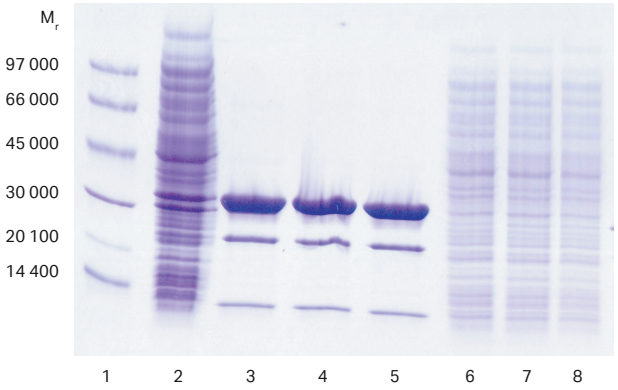


Fig 8. Automated purification of dual-tagged fluorescent protein $(His)_6$-mCherry-Strep(II) using two-step affinity on StrepTrap HP 1 ml (AC 1 eluted peak shown) and HisTrap HP 1 ml (AC 2 whole run shown).

Figure 8 shows the chromatograms and running conditions for each purification, and Figure 9 the SDS-PAGE analysis of the three purified fractions collected from the 1 ml HisTrap HP column.

The results for the three purifications were very similar regarding yield and purity of the dual-tagged protein, thus demonstrating the high reproducibility of this automated two-step affinity purification procedure.

In addition to the target protein at M_r 31 000, Figure 9 shows two contaminants at approx. M_r 10 000 and 21 000, respectively. These may be due to fragmentation of the target protein during SDS-PAGE analysis. The acylimine linkage of the fluorescent chromophore MYG (aa 85-87) may hydrolyze under harsh treatment such as SDS denaturing and boiling (references 1, 2). Cleavage of $(His)_6$-mCherry-Strep(II) between F(84) and the MYG chromophore yields an N-terminal fragment of M_r 9558 and a C-terminal fragment of 20 741. The full-length target protein and the M_r 21 000 fragment were confirmed by MS-analysis.



Lane

1. Low molecular weight markers
2. Start material, *E. coli* lysate with $(His)_6$-mCherry-Strep(II)
3. Run 1, eluted pool
4. Run 2, eluted pool
5. Run 3, eluted pool
6. Run 1, flowthrough
7. Run 2, flowthrough
8. Run 3, flowthrough

Fig 9. SDS-PAGE analysis (reduced conditions) of the three different automated two-step affinity purification of $(His)_6$-mCherry-Strep(II).

Acknowledgement

We thank Martina Nilsson, Robert Svensson and Erik Holmgren, Biovitrum, Stockholm, Sweden, for fruitful discussions and excellent application work.

Further information

Refer to IBA GmbH, Germany (www.iba-go.com) for expression, detection and/or assays for Strep(II)-tagged proteins.

For other information visit www.cytiva.com/hitrap and www.cytiva.com/protein-purification or contact your local Cytiva representative.

References

1. Quillin *et al.*, *Biochemistry*, **44**, 5774–5787, (2005).
2. Shkrob *et al.*, *Biochem. J.*, **392**, 649–654, (2005).

Ordering information

Product	Quantity	Code number
StrepTactin Sepharose High Performance	10 ml	28-9355-99
StrepTactin Sepharose High Performance	50 ml	28-9356-00
StrepTrap HP	1 × 1 ml	29-0486-53
StrepTrap HP	5 × 1 ml	28-9075-46
StrepTrap HP	1 × 5 ml	28-9075-47
StrepTrap HP	5 × 5 ml	28-9075-48

Product	Quantity	Code number
HisTrap HP	1 × 1 ml	29-0510-21
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

* Pack size available by special order

Empty lab-scale columns

Product	Quantity	Code number
Tricorn 5/20 column, 5 mm i.d.	1	18-1163-08
Tricorn 5/50 column, 5 mm i.d.	1	18-1163-09
Tricorn 10/20 column, 10 mm i.d.	1	18-1163-13
Tricorn 10/50 column, 10 mm i.d.	1	18-1163-14
Tricorn 10/100 column, 10 mm i.d.	1	18-1163-15
XK 16/20 column, 16 mm i.d.	1	18-8773-01
XK 26/20 column, 26 mm i.d.	1	18-1000-72

Accessories	Quantity	Code number
1/16″ male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16″ female/M6 male	6	18-1112-57
Union M6 female/1/16″ male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16″ male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16″†	5	11-0004-64
Fingertight stop plug, 1/16″‡	5	11-0003-55

* One connector included in each HiTrap package
† Two, five, or seven stop plugs female included in HiTrap packages depending on products.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Code number
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Columns and Media, Selection guide	18-1121-86
HiTrap Column guide	18-1129-81
Prepacked chromatography columns for ÄKTA systems	28-9317-78

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