TALON Superflow and prepacked formats

AFFINITY CHROMATOGRAPHY

TALON® Superflow™ is a cobalt-based immobilized metal affinity chromatography medium (IMAC) offering enhanced selectivity for histidine-tagged proteins compared to nickel-charged media. TALON Superflow is available in 10 ml and 50 ml lab packs, and prepacked in a range of columns and 96-well plates. Prepacked HiTrap™ TALON crude, His GraviTrap™ TALON, His SpinTrap™ TALON, and His MultiTrap™ TALON (Fig 1) enable different throughput and scales from screening in low microgram scale to milligram preparative purification of histidine-tagged recombinant proteins by IMAC.

Key features:

- Suitable for IMAC screening and purification of histidine-tagged proteins when Ni^{2+} is not the optimal choice of metal ion
- Compatible with commonly used IMAC reagents and appropriate for purifying proteins under native or denaturing conditions
- HiTrap TALON crude and His GraviTrap TALON save time by allowing the application of unclarified samples directly, minimizing labor and degradation of proteins
- His SpinTrap TALON and His MultiTrap TALON enable screening with highly reproducible results column-to-column and well-to-well
- High purity in one step using a mild purification process that preserves structure and function of sensitive histidine-tagged proteins

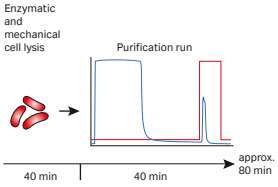
Fig 1. HiTrap TALON crude columns, His GraviTrap TALON columns, His SpinTrap TALON columns, His MultiTrap 96-well plates, and TALON Superflow lab packs.

Description

Direct loading of crude lysates

All columns and 96-well plates prepacked with TALON Superflow allow for rapid screening, purification, and easy scale-up, with a minimum of sample preparation and equipment. Figure 2 illustrates time saved by simplified sample preparation through direct loading of unclarified samples.

Crude protocol



Conventional protocol

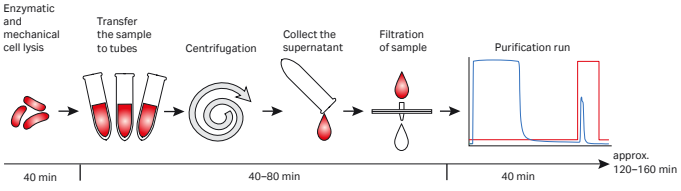


Fig 2. Schematic workflow showing the time saved in purifying histidine-tagged proteins using the crude protocol for HiTrap TALON crude and His GraviTrap TALON columns compared to conventional protocol.

The filters in the top and bottom of the columns and the bottom filter in the 96-well plate make it possible to directly load unclarified lysates without causing back pressure problems or leakage of the TALON Superflow beads. Prior to loading, the cell lysate should be prepared using enzymatic extraction buffers or mechanical methods such as sonication, homogenization, or freeze/thaw. Samples from several different expression systems can be applied directly to HiTrap TALON crude and His GraviTrap TALON columns after thorough cell disruption – without precentrifugation and filtration.

His SpinTrap TALON and His MultiTrap TALON both allow direct loading of unclarified samples. However, clarification is recommended to achieve highest purity and recovery.

TALON Superflow

TALON Superflow chromatography medium consists of highly cross-linked agarose beads with an immobilized chelating group. The characteristics of TALON Superflow are summarized in Table 1. The TALON ligand is a tetradentate chelator charged with Co^{2+} ions. The medium binds polyhistidine-tagged proteins with high selectivity and exhibits a reduced affinity for host proteins giving lower background. TALON Superflow allows protein purification under native or denaturing conditions and can be used with prokaryotic and eukaryotic expression systems. The medium is suitable for batch purification and can also be used for packing into liquid chromatography columns such as Tricorn™ or XK columns.

TALON Superflow chromatography medium is compatible with commonly used aqueous buffers, denaturants, and a range of other additives (Table 2).

Table 1. TALON Superflow characteristics

Matrix	Cross-linked agarose, 6%
Precharged ion	Cobalt
Particle size distribution	60 to 160 µm
Binding capacity[1]	up to 20 mg histidine-tagged protein/ml medium
Maximum linear flow rate[2]	2000 cm/h
pH stability[3, 4]	
Cleaning[5]	2 to 14
Working[6]	3 to 12
Storage	20% ethanol at 4ºC to 8ºC
Compatibility during use	Stable in all commonly used buffers, detanurants, and detergents (see Table 2)

[1] The binding capacity for individual proteins may vary.
[2] H_2O in a 0.75 × 10 cm (i.d. × H) column.
[3] Co^{2+}-stripped medium.
[4] Below pH 4, metal ions will be stripped off the medium, and therefore neutral to slightly alkaline pH (pH 7 to 8) is recommended.
[5] Refers to the pH interval for regeneration.
[6] 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. Compatible reagents for TALON Superflow[1, 2]

Reagent	Acceptable concentration
β-Mercaptoethanol[3]	10 mM (with caution)
CHAPS, SDS, sarcosyl[4]	1% (with caution)
Ethanol[5]	30%
Ethylene glycol	30%
HEPES	50 mM
Glycerol	20%
Guanidinium hydrochloride	6 M
Imidazole[6]	≤ 500 mM at pH 7.0 to 8.0, for elution
KCl	500 mM
MES	20 mM
MOPS	50 mM
Sodium chloride	1.0 M
NP-40	1%
Tris[7]	50 mM
Triton™-X 100	< 1%
Urea	8 M

[1] Data provided by Clontech Laboratories, Inc.
[2] EDTA and other chelators, such as EGTA, will strip Co^{2+} ions from the medium; EDTA may be used, but must be removed prior to sample application. Strong reducing agents should also be avoided (e.g., DTT, DTE, and TCEP) because they interfere with Co^{2+} ions binding to the medium.
[3] Use TALON Superflow immediately after equilibrating with buffers containing β-Mercaptoethanol, otherwise the medium will change color. Do not store the medium in buffers containing β-Mercaptoethanol.
[4] Ionic detergents like CHAPS (3-[{3-Cholamidopropyl}-dimethylammonio]-1-propane-sulfonate), SDS (sodium dodecyl sulfate), and sarcosyl are compatible up to 1%. However, due to their charged nature, you should anticipate interference with binding.
[5] Ethanol may precipitate proteins, causing low yields and column clogging.
[6] Imidazole at concentrations higher than 5 to 10 mM may cause lower yields of histidine-tagged proteins because it competes with the histidine side chains (imidazole groups) for binding to the immobilized metal ions.
[7] Tris coordinates weakly with metal ions, causing a decrease in capacity.

HiTrap TALON crude columns

HiTrap TALON crude columns can be operated with a syringe, peristaltic pump, or chromatography system such as ÄKTA™ systems. The columns are made of biocompatible polypropylene that does not interact with biomolecules. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Note that HiTrap TALON crude columns cannot be opened or refilled. The characteristics of HiTrap TALON crude are summarized in Table 3.

Table 3. HiTrap TALON crude characteristics

Column volume	1 ml and 5 ml
Column dimensions, i.d. × H	0.7 × 2.5 cm (1 ml column) 1.6 × 2.5 cm (5 ml column)
Recommended flow rate[1]	1 ml/min (1 ml column) 5 ml/min (5 ml column)
Maximum flow rate[1]	4 ml/min (1 ml column) 20 ml/min (5 ml column)
Column hardware pressure limit[2]	5 bar (0.5 MPa, 70 psi)
Chromatography medium	TALON Superflow (see Table 1)

[1] H_2O at room temperature.
[2] The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium and the column tubing used.

His SpinTrap TALON columns

His SpinTrap TALON columns (Fig 3) are excellent for screening of expression levels and purification conditions prior to scale up. The columns are designed for use in a microcentrifuge (Fig 4), and Figure 5 shows the rapid purification procedure using His SpinTrap TALON, taking approximately 10 min. The prepacked columns enable highly reproducible results column-to-column, with a relative sd < 10% based on measurements of recovery and purity (results not shown). Each column contains 100 µl of TALON Superflow, enough for purifying up to 1 mg of histidine-tagged protein. Each package contains 50 columns. Characteristics of His SpinTrap TALON are summarized in Table 4.

Fig 3. His SpinTrap TALON columns.

Fig 4. His SpinTrap TALON columns are designed for efficient, small-scale purification up to 1 mg of histidine-tagged proteins.

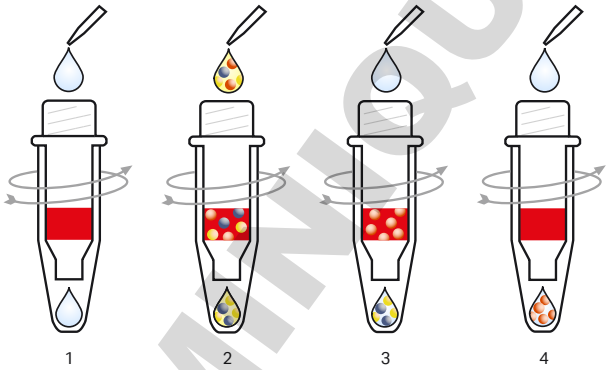


Fig 5. Purifying histidine-tagged proteins with His SpinTrap TALON is a simple four-stage procedure that can be performed in 10 min using a microcentrifuge: 1) After placing the column in a 2 ml microcentrifuge tube, equilibrate by adding binding buffer and centrifuge. 2) Add sample, centrifuge. 3) Wash with binding buffer, centrifuge. 4) Elute the target protein with elution by centrifugation

His MultiTrap TALON 96-well plates

His MultiTrap TALON 96-well filter plates (Fig 6) are prepacked with 50 µl TALON Superflow per well and provide highly reproducible high-throughput screening and rapid small-scale purification of histidine-tagged proteins from clarified samples. Typical applications include expression screening of different constructs, screening for solubility of proteins, and optimization of the conditions for small-scale parallel purification. Purification of up to 1 mg of histidine-tagged proteins per well. The 96-well plate format gives great flexibility, both when working with automated robotic systems and when manually using centrifugation or vacuum. Consistent well-to-well and plate-to-plate performance ensures high reproducibility with a relative sd < 10% based on measurements of recovery and purity (results not shown). Characteristics of His MultiTrap TALON are summarized in Table 4.

Fig 6. His MultiTrap TALON 96-well plates.

His GraviTrap TALON columns

His GraviTrap TALON columns (Fig 7) are prepacked with 1 ml TALON Superflow and provide simple manual purification of up to 15 mg of histidine-tagged proteins. The different purification steps are performed using gravity flow and require no further equipment (Fig 8). Large sample volumes can be applied in one go, and the histidine-tagged protein is effectively eluted in a small volume. Each package contains 10 prepacked columns and each column is delivered in a package that converts into a column stand (Workmate). The plastic tray in the product package can be used to collect liquid waste. The columns are manufactured from biocompatible polypropylene, and special frits in each column protect the medium from running dry during purification. Connecting Labmate™ PD-10 Buffer Reservoir to the column increases the loading capacity from 10 ml to approximately 35 ml. The characteristics of His GraviTrap TALON are summarized in Table 4.

Fig 7. His GraviTrap TALON columns.

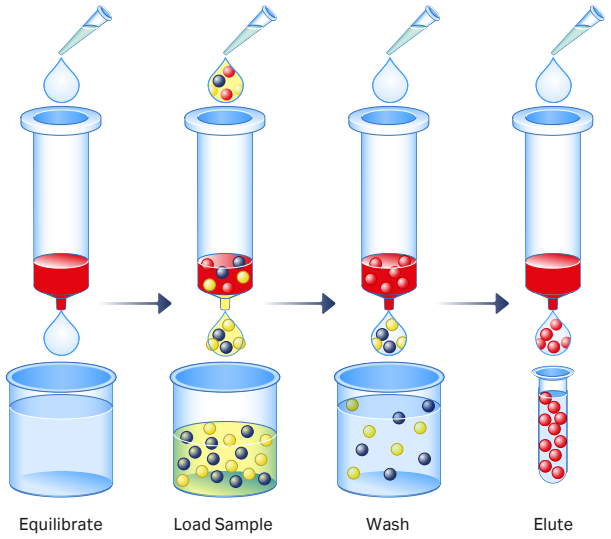


Fig 8. A fast and simple four-stage process for purifying histidine-tagged proteins on His GraviTrap TALON.

Table 4. His GraviTrap TALON, His SpinTrap TALON and His MultiTrap TALON characteristics

Column and 96-well plate material	Polypropylene barrel and plate, polyethylene frits
Protein binding capacity[1]	
- His GraviTrap TALON	Up to 15 mg histidine-tagged protein/column
- His SpinTrap TALON	Up to 1 mg histidine-tagged protein/column
- His MultiTrap TALON	Up to 1 mg histidine-tagged protein/well
Bed volume in columns/wells	
- His GraviTrap TALON	1 ml/column
- His SpinTrap TALON	100 µl/column
- His MultiTrap TALON	50 µl/well (500 µl of 10% slurry)
Total volume in columns/wells	
- His GraviTrap TALON	13.5 ml
- His SpinTrap TALON	1000 µl
- His MultiTrap TALON	800 µl
Reproducibility column-to column, plate-to-plate and well-to-well	± 10%
Filter plate size of His MultiTrap TALON	127.8 × 85.5 × 30.6 mm according to ANSI/SBS 1-2004, 3-2004 and 4-2004 standards
Number of wells	96
Avoid in buffers	Chelating agents, e.g., EDTA, EGTA, citrate and DTT, DTE, and TCEP

[1] The binding capacity for individual proteins may vary.

Applications

Yield and purity using unclarified vs clarified sample

HiTrap TALON crude columns simplify purification by eliminating the need for precentrifugation and filtration steps. For comparison, unclarified and clarified (centrifugation at 30 000 × g for 20 min) *E. coli* extracts containing the histidine-tagged protein GEHC1-$(His)_6$ were loaded on HiTrap TALON crude 1 ml columns. After purification, purity (analyzed by SDS-PAGE) and yield (calculated from absorbance measurements) of eluted fraction pools were determined. Figure 9 shows that purification using unclarified extract was similar to when clarified sample was used. The amount of GEHC1-$(His)_6$ eluted was 13 and 12 mg when loading unclarified and clarified sample, respectively. In addition, the purity did not significantly differ and SDS-PAGE analysis showed high purity for both samples (> 90%; Fig 10). By eliminating the need for precentrifugation and filtration, HiTrap TALON crude columns save approximately 40 min.

Column: HiTrap TALON crude 1 ml
Sample: Unclarified and clarified *E. coli* lysate containing GEHC1-$(His)_6$ (M_r 47 000)
Sample volume: 20 ml
Binding/wash buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4
Flow rate: 1 ml/min
Detection: Absorbance, 280 nm
System: ÄKTA

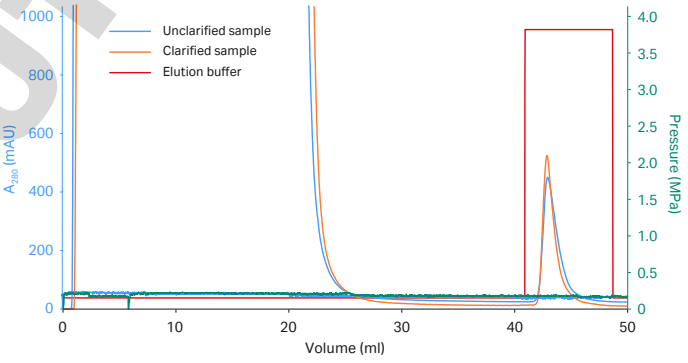
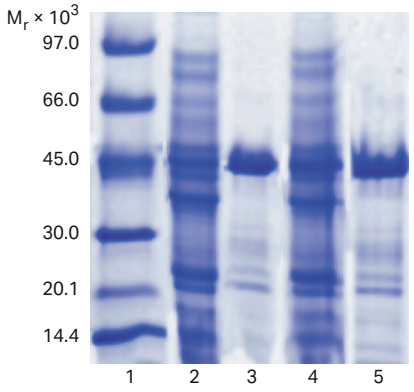


Fig 9. Comparison study loading unclarified (blue line) and clarified (orange line) *E. coli* samples containing the histidine-tagged protein GEHC1-$(His)_6$ on HiTrap TALON crude. Overlay of absorbance curves.



1. LMW-SDS Marker Kit
2. Start material, unclarified
3. Pool purified protein, unclarified
4. Start material, clarified
5. Pool purified protein, clarified

Fig 10. SDS-PAGE analysis (reducing conditions, ExcelGel™ SDS Gradient 8-18, Coomassie™ stained) of pools containing purified GEHC1-$(His)_6$. Comparison of runs performed on HiTrap TALON crude using unclarified and clarified sample.

Repeated purification runs possible without regeneration of HiTrap TALON crude

Reproducibility of HiTrap TALON crude columns in terms of purity and recovery over a number of repeated runs without stripping, cleaning, or Co^{2+} recharging was investigated. In this study, GEHC1-$(His)_6$ was purified from an unclarified lysate of *E. coli* in four consecutive runs (Fig 11). The yield (calculated from absorbance measurements) was reproducible, as shown in Figure 12, and the back pressure did not increase during the four purification runs. The purity, determined by SDS-PAGE analysis, was high for all purification cycles (> 90%; Fig 13).

Column:	HiTrap TALON crude 1 ml
Sample:	Unclarified *E.coli* lysate containing GEHC1-$(His)_6$ (M_r 47 000), prepared by homogenization
Sample volume:	20 ml
Binding/wash buffer:	50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4
Flow rate:	1 ml/min
System:	ÄKTA
Detection:	Absorbance, 280 nm

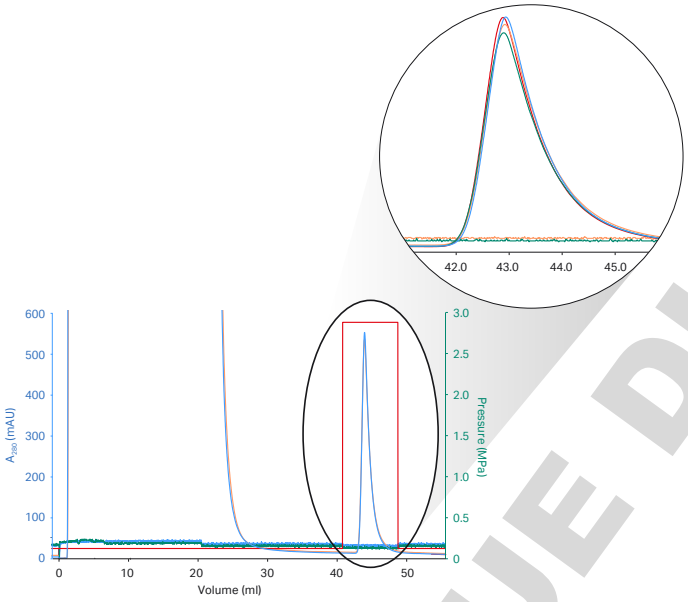


Fig 11. Overlaid chromatograms from four consecutive purification cycles loading unclarified *E.coli* lysate containing GEHC1-$(His)_6$ on HiTrap TALON crude without cleaning and regeneration in between cycles. Pressure curves for run 1 (green) and run 4 (orange) are also shown. A close-up of the eluted peak shows high reproducibility between the runs.

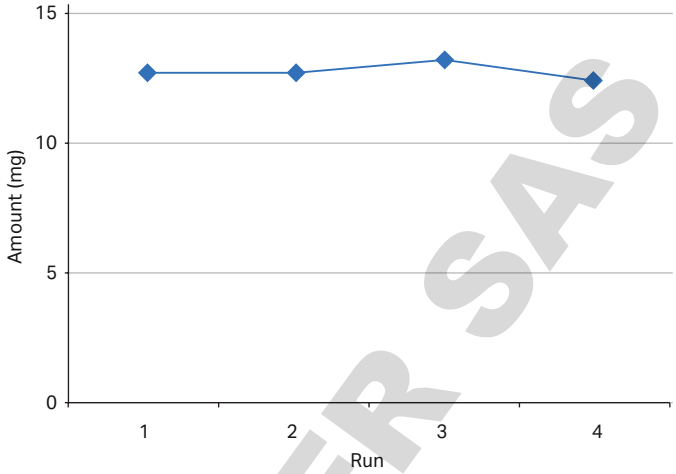
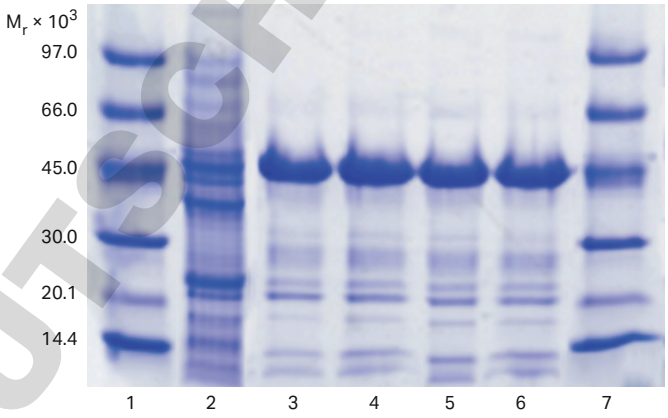


Fig 12. Four purification cycles, run consecutively without regenerating the medium in between cycles, gave consistent and reproducible yields.



1. LMW-SDS Marker Kit
2. Start material
3. Pool elution, run 1
4. Pool elution, run 2
5. Pool elution, run 3
6. Pool elution, run 4
7. LMW-SDS Marker Kit

Fig 13. SDS-PAGE analysis (reducing conditions, ExcelGel SDS Gradient 8-18, Coomassie stained) of pools containing purified GEHC1-$(His)_6$ from four consecutive purification runs on a HiTrap TALON crude 1 ml column. High purity was obtained for all four purification runs performed on HiTrap TALON crude without recharging and cleaning in between runs.

Increased purity of a kinase using gradient elution

The imidazole concentration in binding and wash buffer is critical for obtaining as high purity and yield as possible. The optimal imidazole concentration depends on the protein, and increasing imidazole concentration will increase purity but can also decrease the yield. Normally, low concentrations of imidazole are used in binding/wash buffers and in the sample, to minimize binding of host cell proteins.

Elution by using an imidazole gradient is an efficient approach for obtaining high purity of histidine-tagged proteins. This is clearly demonstrated in a study of a histidine-tagged kinase, ERK3-$(His)_6$, purified on HiTrap TALON crude 1 ml. Two different purification approaches were used, linear gradient elution and step elution (Fig 14A and 14B, respectively). No optimization was performed prior to the step elution. Fractions were analyzed by SDS-PAGE (Fig 14C). Figure 14C shows that the eluted fraction from the step elution contained impurities, while eluted fractions from the gradient elution demonstrated increased purity.

Column:	HiTrap TALON crude 1 ml
Sample:	Unclarified *E.coli* BL21 containing ERK3-$(His)_6$ (M_r 38 300), prepared by sonication
Sample volume:	20 ml
Binding/wash buffer:	50 mM NaH_2PO_4, 300 mM NaCl, pH 7.4
Elution buffer:	50 mM NaH_2PO_4, 300 mM NaCl, pH 7.4 (A): Gradient, 0 to 150 mM imidazole (B): Step, 150 mM imidazole
Flow rate:	1 ml/min
System:	ÄKTA
Detection:	Absorbance, 280 nm

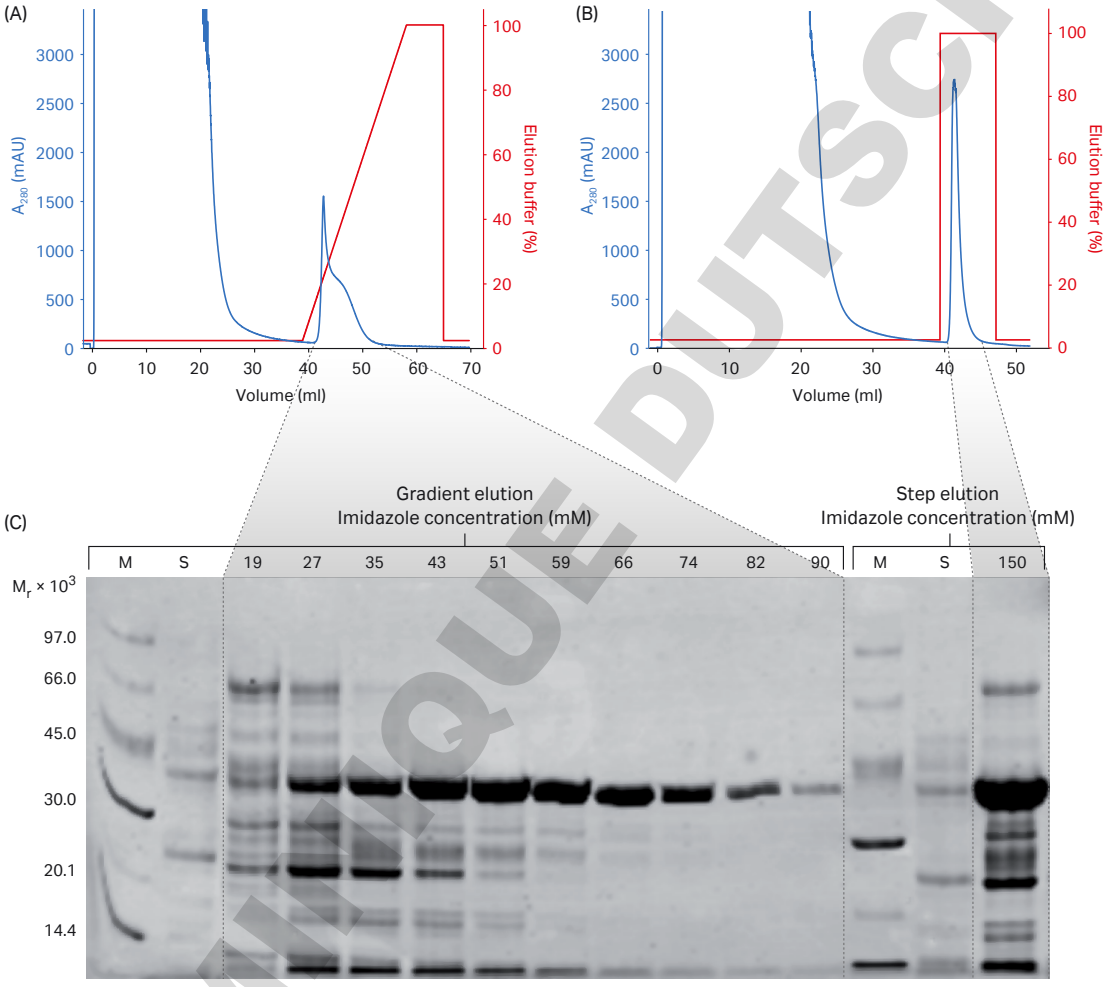


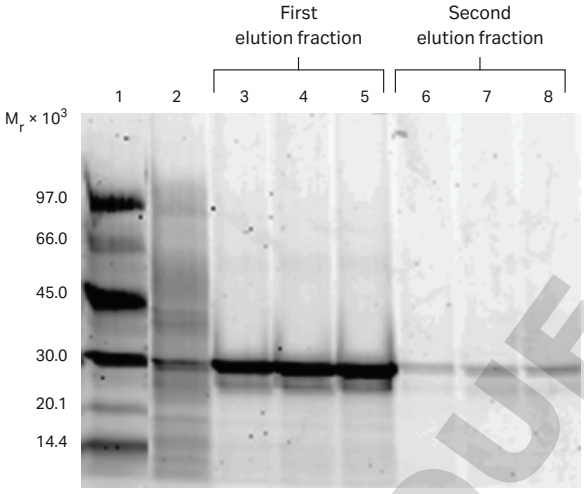
Fig 14. Purification of ERK3-$(His)_6$ in unclarified *E.coli* sonicate using HiTrap TALON crude 1 ml. (A) Protein was eluted using a 20 column volume (CV) linear imidazole gradient (0 to 150 mM imidazole). (B) Protein was eluted using 8 CV step elution (150 mM imidazole). (C) SDS-PAGE analysis (reducing conditions, ExcelGel SDS Gradient 8-18, Deep Purple stained) of start material and eluted fractions (M = LMW-SDS Marker Kit; S = start material). The gel was scanned using Ettan™ DIGE Imager fluorescence scanner. The imidazole concentration during elution is shown for each eluate.

Simple and rapid protein purification using His GraviTrap TALON

His GraviTrap TALON enables fast and easy manual purification of histidine-tagged proteins without the need of a purification system. Using a gravity-flow protocol, histidine-tagged green fluorescent protein (GFP-His) added to *E. coli* lysate was purified with His GraviTrap TALON in less than 30 min (Table 5). The recovery was calculated using absorbance measurements and was found to be 95%, with 96% of the purified protein eluting in the first elution fraction. Figure 15 shows the SDS-PAGE analysis of the first and second elution fractions. The results indicate a purity of ≥ 93% for the purified GFP-His protein.

Table 5. Experimental conditions for His GraviTrap TALON

Column	His GraviTrap TALON
Sample	GFP-His (1 mg/ml) added to *E. coli* lysate, prepared by enzymatic lysis and sonication
Sample volume	8 ml
Binding buffer	50 mM NaH_2PO_4, 300 mM NaCl, pH 7.4
Wash buffer	50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4



1. LMW-SDS Marker Kit
2. Start material, diluted 50-fold
3. Replicate 1
4. Replicate 2
5. Replicate 3
6. Replicate 1
7. Replicate 2
8. Replicate 3

Fig 15. SDS-PAGE analysis of elution fractions from a purification of GFP-His (M_r 28 000) added to *E. coli* lysate. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant™ TL software. The experiment was performed in triplicate.

Comparison of purity achieved using various gravity flow columns

A comparative analysis of the recovery and purity achieved when purifying GFP-His added to *E. coli* lysate was performed at Cytiva laboratories in order to investigate the differences in performance between three different gravity flow columns. His GraviTrap TALON (TALON Superflow medium) and His GraviTrap (Nickel Sepharose 6 Fast Flow medium), both from Cytiva, were compared with HisPur™ Cobalt Spin Column from Thermo Scientific (Table 6). Purification of clarified GFP-His added to *E. coli* lysate was executed in triplicate according to each manufacturer's gravity protocol, and the results showed that the recovery was high at ≥ 95% for all columns. Figure 16 displays the degree of purity estimated by SDS-PAGE analysis of the eluted fraction containing the most purified protein, showing that the highest purity was achieved using His GraviTrap TALON.

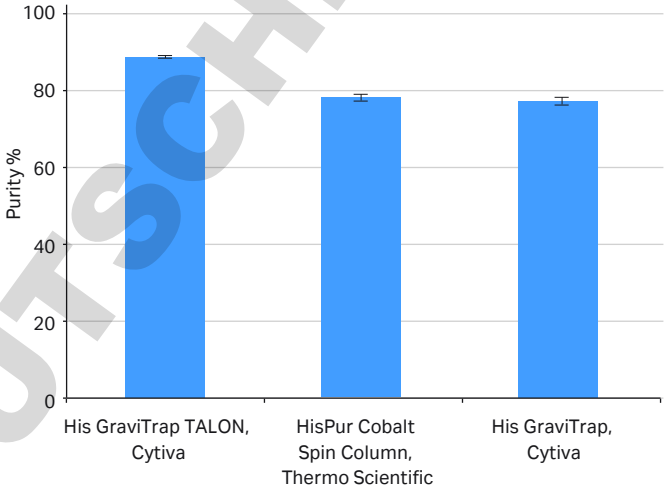
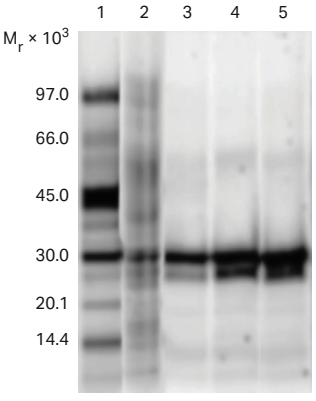


Fig 16. Purification of GFP-His added to *E. coli* lysate with three replicates using three different gravity-flow columns. SDS-PAGE gel electrophoresis was used to determine the degree of purity (Fig 17). Using the Student's t-test, the differences in purity between His GraviTrap TALON and His GraviTrap and HisPur Cobalt Spin Column respectively were statistically significant ($p < 0.05$, df = 4). The standard error of the mean is shown as error bars.



1. LMW-SDS Marker Kit
2. Start material, diluted 50-fold
3. His GraviTrap TALON, Cytiva
4. HisPur Cobalt spin column, Thermo Scientific
5. His GraviTrap, Cytiva

Fig 17. SDS-PAGE analysis of elution fractions from the purification of GFP-His (M_r 28 000) added to *E. coli* using three different gravity-flow columns. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

Table 6. Experimental conditions for purification using gravity flow columns from Cytiva and Thermo Scientific

Supplier	Cytiva	Cytiva	Thermo Scientific
Column	His GraviTrap TALON	His GraviTrap	HisPur Cobalt Spin Column
Medium volume	1 ml	1 ml	1 ml
Sample	GFP-His (0.5 mg/ml) added to *E. coli* lysate, prepared by enzymatic lysis and sonication followed by clarification through centrifugation		
Sample volume	10 ml	10 ml	10 ml
Binding buffer	50 mM NaH_2PO_4, 300 mM NaCl, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 7.4
Wash buffer	50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 7.4
Elution buffer	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 500 mM imidazole, pH 7.4	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4

Repeatable purification using His SpinTrap TALON

One advantage of His SpinTrap TALON is the possibility to perform several purifications both simultaneously and with high repeatability. In order to investigate the repeatability, unclarified GFP-His added to *E. coli* lysate was loaded onto ten His SpinTrap TALON columns and centrifuged (Table 7). Figure 18 shows high purity (> 90%) of the eluted GFP-His. Recovery was calculated with absorbance measurements and found to be highly repeatable with an average of 71% recovered and a relative standard deviation (RSD) of 6%.

Table 7. Experimental conditions for His SpinTrap TALON

Column	His SpinTrap TALON
Sample	Unclarified GFP-His (1 mg/ml) added to *E. coli* lysate, prepared by enzymatic lysis and sonication
Sample volume	500 µl
Binding buffer	50 mM NaH_2PO_4, 300 mM NaCl, pH 7.4
Wash buffer	50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4
Centrifugal force	100 × g for 30 s
System	Centrifuge 5415R Eppendorf

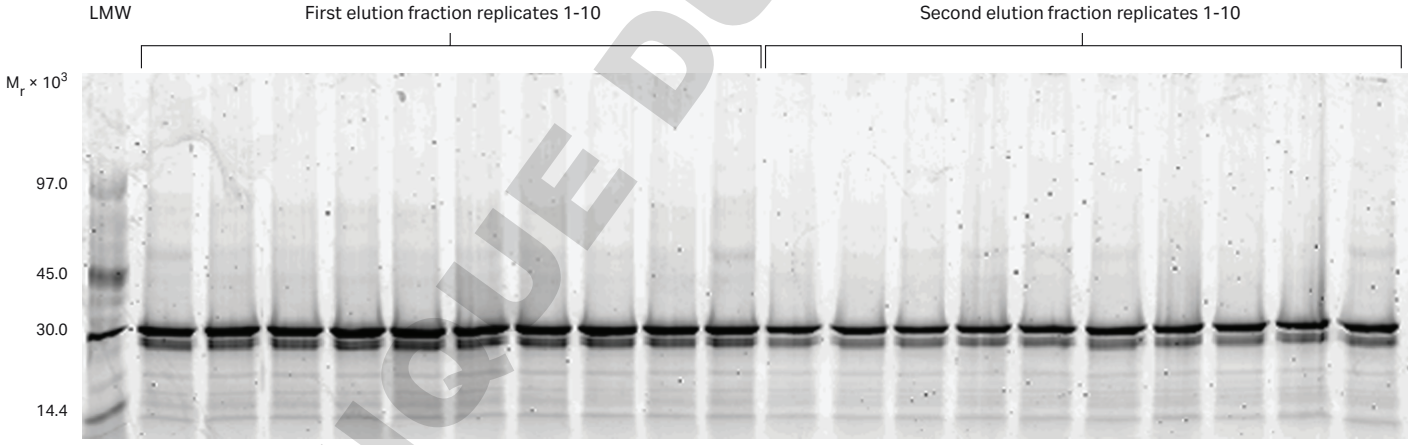
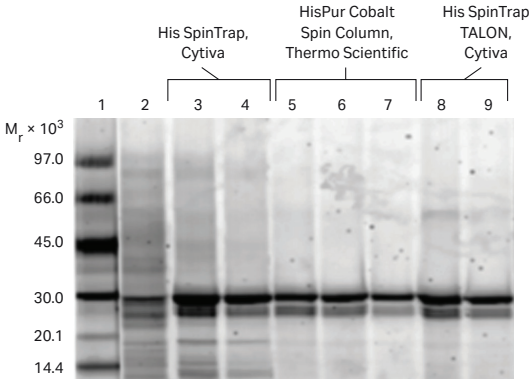


Fig 18. SDS-PAGE analysis of elution fractions from ten replicate purification runs of GFP-His (M_r 28 000) using His SpinTrap TALON. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software. LMW = LMW-SDS Marker Kit.

Comparison of His SpinTrap, His SpinTrap TALON, and HisPur Cobalt Spin Columns

To investigate the differences in performance between three different spin columns, a comparative analysis was performed at Cytiva laboratories with regards to recovery and purity achieved when purifying GFP-His added to *E. coli* lysate. His SpinTrap TALON (TALON Superflow medium) and His SpinTrap (Nickel Sepharose 6 Fast Flow medium), both from Cytiva, were compared with HisPur Cobalt Spin Column from Thermo Scientific (Table 8). Purification of clarified GFP-His added to E. coli lysate was performed according to each manufacturer's protocol, and the results displayed in Figure 20A show that the highest recovery was achieved using His SpinTrap (93%), whereas His SpinTrap TALON and HisPur Cobalt Spin Column gave a recovery of 82% and 76% respectively. Figure 20B shows that the purity was approximately equal for His SpinTrap TALON and His Cobalt Spin Column (85% and 83% respectively), whereas it was significantly lower (70%) for His SpinTrap.



1. LMW-SDS Marker Kit
2. Start material, diluted 50-fold
3. Elution 1
4. Elution 2
5. Elution 1
6. Elution 2
7. Elution 3
8. Elution 1
9. Elution 2

Fig 19. SDS-PAGE analysis of elution fractions from the purification of GFP-His (M$_r$ 28 000). The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

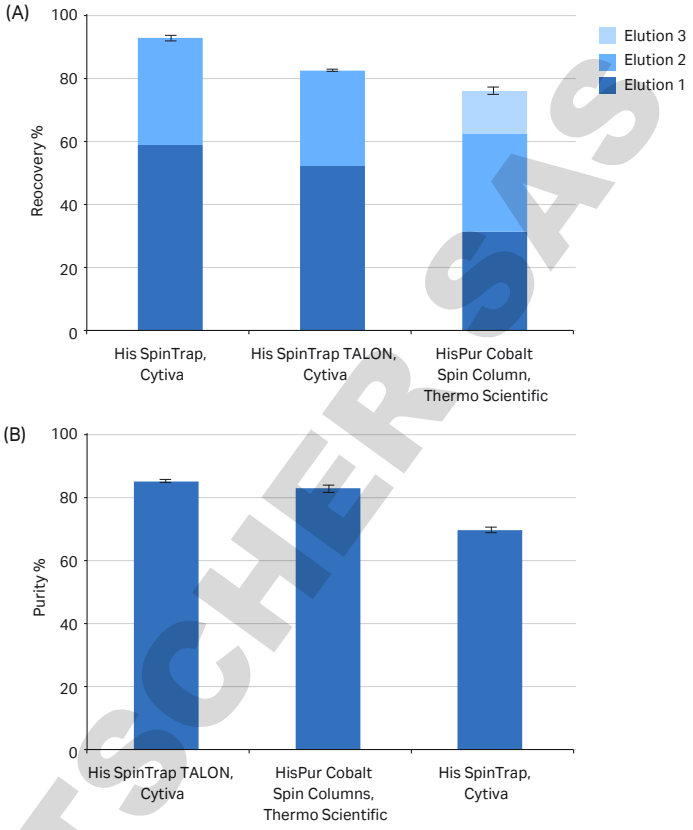


Fig 20. GFP-His added to *E. coli* lysate using three different spin columns. 500 µl GFP-His was loaded onto each column and the experiment was performed in triplicate. (A) Recovery was calculated using absorbance measurements. The colors of the bars represent the amount of protein eluted in each fraction. In accordance with the protocol supplied from Thermo Scientific, elution was performed three times for the HisPur Cobalt Spin Columns. (B) SDS-PAGE gel electrophoresis was used to determine purity (Fig 19). Using Student's t-test, the differences in recovery between His SpinTrap TALON and His SpinTrap and HisPur Cobalt Spin Column respectively were statistically significant ($p < 0.05$, df = 4). Using the same test with regards to purity confirmed statistically significant differences between His SpinTrap TALON and His SpinTrap, but not between His SpinTrap TALON and HisPur Cobalt Spin Column. The standard error of the mean is shown as error bars.

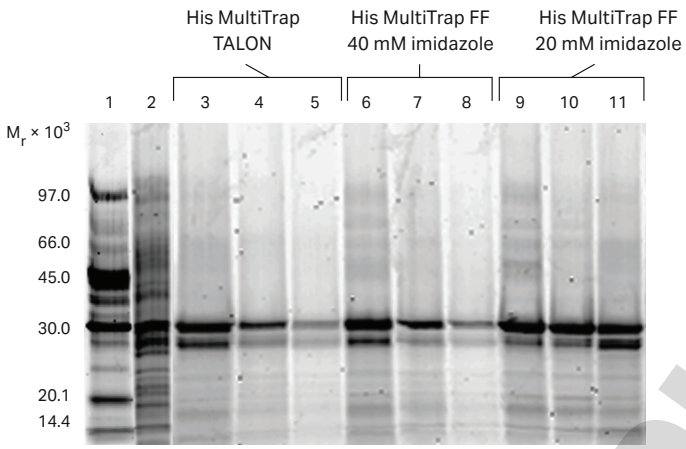
Table 8. Experimental conditions for purification using spin columns from Cytiva and Thermo Scientific

Supplier	Cytiva	Cytiva	Thermo Scientific
Column	His SpinTrap TALON	His SpinTrap	HisPur Cobalt Spin Column
Medium volume	100 µl	100 µl	200 µl
Sample	GFP-His (1 mg/ml) added to *E. coli* lysate, prepared by enzymatic lysis and sonication followed by clarification through centrifugation		
Sample volume	500 µl	500 µl	500 µl
Binding buffer	50 mM NaH_2PO_4, 300 mM NaCl, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 7.4
Wash buffer	50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 7.4
Elution buffer	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 500 mM imidazole, pH 7.4	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4
Centrifugal force	100 × g for 30 s	100 × g for 30 s	700 × g for 2 min
System	Centrifuge 5415R Eppendorf	Centrifuge 5415R Eppendorf	Centrifuge 5415R Eppendorf

Comparison of Cobalt and Nickel IMAC media using His MultiTrap TALON and His MultiTrap FF

In any comparison between different purification media, recovery and purity depend on the protein to be purified. Keeping this in mind, one of the benefits of Cobalt IMAC medium compared to Nickel IMAC medium is that it often results in higher purity, even though it uses milder conditions during both purification and elution. Nickel IMAC medium, on the other hand, often delivers better recovery at the cost of harsher purification conditions.

To investigate the differences in performance between 96-well plates with Nickel IMAC medium and Cobalt IMAC medium, multiple purifications were performed using His MultiTrap FF (Nickel Sepharose 6 Fast Flow medium) with two separate protocols differing in binding buffer imidazole concentration, and His MultiTrap TALON (TALON Superflow medium) using no imidazole in the binding buffer.



1. LMW-SDS Marker Kit
2. Start material
3. Elution 1
4. Elution 2
5. Elution 3
6. Elution 1
7. Elution 2
8. Elution 3
9. Elution 1
10. Elution 2
11. Elution 3

Fig 21. SDS-PAGE analysis of elution fractions from the purification of GFP-His (M_r 28 000) using His MultiTrap TALON and His MultiTrap FF. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

During the elution, the imidazole concentration was 500 mM for His MultiTrap FF and 150 mM for His MultiTrap TALON. GFP-His added to *E. coli* lysate was loaded onto the wells of the His MultiTrap FF plate and the His MultiTrap TALON plate respectively (Table 9). The results presented in Figure 22 show that the highest recovery was achieved with His MultiTrap FF using the lower concentration of imidazole, whereas the purest elution fractions were obtained with His MultiTrap TALON.

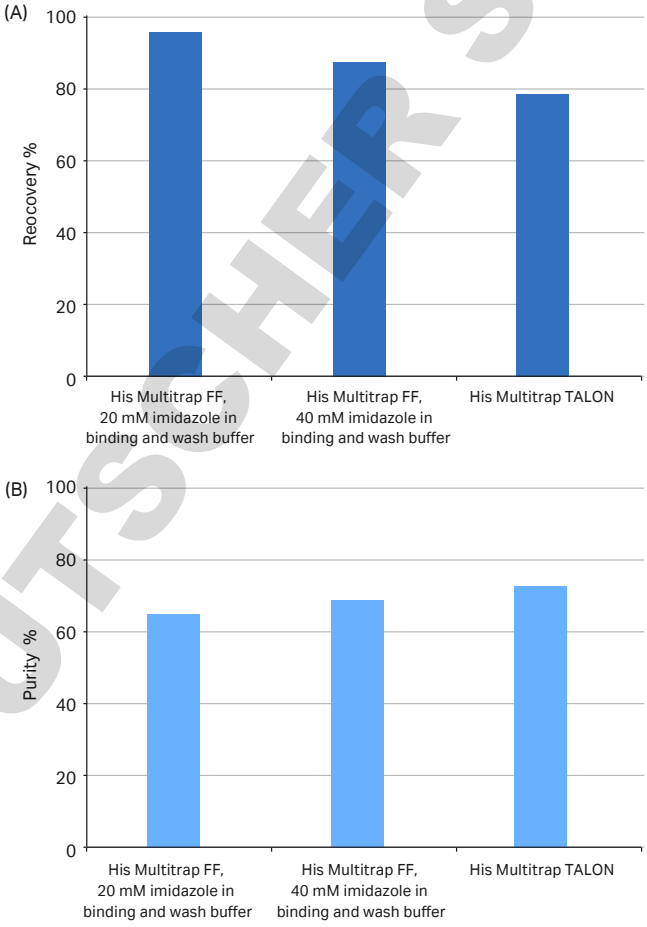


Fig 22. Purification of GFP-His added to *E. coli* lysate using His MultiTrap TALON and His MultiTrap FF. (A) Recovery was calculated based on absorbance measurements, and (B) SDS-PAGE gel electrophoresis was used to determine purity (Fig 21).

Table 9. Experimental conditions for purification using His MultiTrap TALON and His MultiTrap FF

96-well plate	His MultiTrap TALON	His MultiTrap FF	His MultiTrap FF
No of samples	96 (1 plate)	48 (½ plate)	48 (½ plate)
Sample	GFP-His (0.5 mg/ml) added to *E. coli* lysate, prepared by enzymatic lysis and sonication followed by clarification through centrifugation		
Sample volume	500 µl	500 µl	500 µl
Binding buffer	50 mM NaH_2PO_4, 300 mM NaCl, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 40 mM imidazole, pH 7.4
Wash buffer	50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 40 mM imidazole, pH 7.4
Elution buffer	50 mM NaH_2PO_4, 300 mM NaCl, 150 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 500 mM imidazole, pH 7.4	20 mM NaH_2PO_4, 500 mM NaCl, 500 mM imidazole, pH 7.4
Pressure	-0.15 to -0.30 bar	-0.15 to -0.30 bar	-0.15 to -0.30 bar
System	Vacuum regulator IRV3000-FO2 SMC	Vacuum regulator IRV3000-FO2 SMC	Vacuum regulator IRV3000-FO2 SMC

Ordering information

Product	Quantity	Code number
HiTrap TALON crude	1 × 1 ml	29-0485-65
HiTrap TALON crude	5 × 1 ml	28-9537-66
HiTrap TALON crude	100 × 1 ml*	28-9538-05
HiTrap TALON crude	5 × 5 ml	28-9537-67
HiTrap TALON crude	100 × 5 ml*	28-9538-09
TALON Superflow	10 ml	28-9574-99
TALON Superflow	50 ml	28-9575-02
His SpinTrap TALON	50 × 0.1 ml	29-0005-93
His GraviTrap TALON	10 × 1 ml	29-0005-94
His MultiTrap TALON	4 × 96-well plates	29-0005-96

Empty lab-scale columns	Quantity	Code number
Tricorn 5/20 column, 5 mm i.d.	1	28-4064-08
Tricorn 5/50 column, 5 mm i.d.	1	28-4064-09
Tricorn 10/20 column, 10 mm i.d.	1	28-4064-13
Tricorn 10/50 column, 10 mm i.d.	1	28-4064-14
Tricorn 10/100 column, 10 mm i.d.	1	28-4064-15
XK 16/20 column, 16 mm i.d.	1	28-9889-37

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 ÄKTA design	8	28-4010-81
Stop plug female, 1/16"‡	2	11-0004-64
Fingertight stop plug, 1/16"§	5	11-0003-55
LabMate PD-10 Buffer Reservoir	10	18-3216-03
Collection plate (96-well, 500 µl, V-bottom)	5	28-4039-43

* Pack size available by special order.
† One connector is 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
Affinity Chromatography Columns and Media Selection guide	18-1121-86
Prepacked chromatography columns for ÄKTA systems, Selection guide	28-9317-78

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