His Mag **Sepharose** excel
Ni **Sepharose** excel **HisTrap** excel

AFFINITY CHROMATOGRAPHY

His Mag Sepharose™ excel and Ni Sepharose excel (Fig 1) are immobilized metal ion affinity chromatography (IMAC) media (resins) designed for capture and purification of histidine-tagged proteins from various sample types. Nickel ions are very strongly bound to both media, making them especially suitable for purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants. The media significantly simplify and speed up the workflow since they allow direct loading of large sample volumes without removing agents that normally would cause metal ion stripping (Fig 2, 3). The strong nickel ion binding also provides very high resistance to EDTA and reducing agents like DTT.

Key features:

- Load eukaryotic cell culture samples containing secreted histidine-tagged proteins directly with retained binding capacity
- Increase target protein yield and decrease degradation through reduced and simplified sample handling
- Resistant to EDTA (100 mM) and reducing agents such as DTT (5 mM)
- Choose between several different formats for screening and preparative purification of histidine-tagged proteins

Ni Sepharose excel and His Mag Sepharose excel can be successfully used for purification from various sample types, including *E. coli* lysates. However, for samples that do not cause extensive metal ion stripping, such as ordinary *E. coli* lysates, alternative media such as Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance, normally show higher affinity for histidine-tagged proteins.

Fig 1. His Mag Sepharose excel is magnetic beads designed for simple and efficient small-scale purification and screening. Ni Sepharose excel is available for all scales of work from convenient, prepacked HisTrap™ excel columns to bulk quantities.

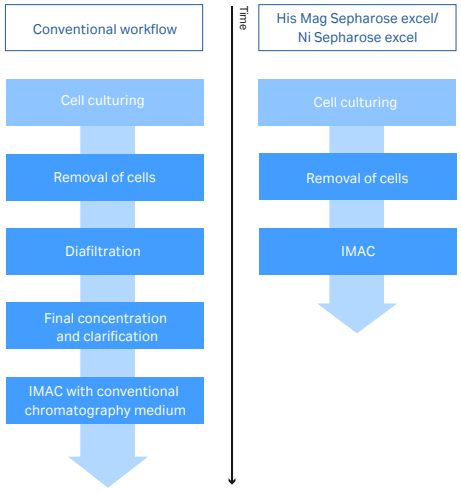


Fig 2. Time is saved using His Mag Sepharose excel or Ni Sepharose excel compared to conventional IMAC workflows.

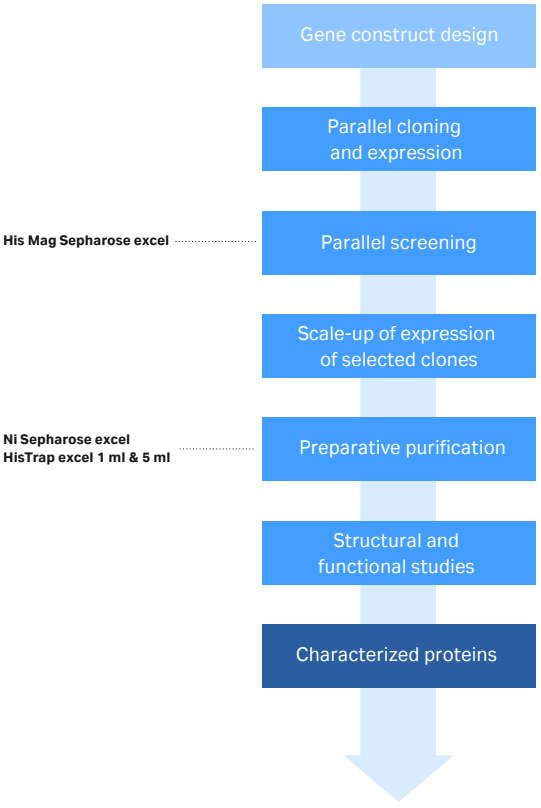


Fig 3. The His Mag Sepharose excel and Ni Sepharose excel products are designed to fit into the protein expression and purification workflow.

Background

IMAC purification of histidine-tagged proteins secreted into eukaryotic cell culture supernatants has traditionally been challenging because of incompatibility between the IMAC medium and the cell culture medium. The cell culture medium strips the immobilized metal ions from the IMAC medium during sample loading, resulting in low or no binding of the target protein. The purification has been further complicated by the fact that the target protein concentration is often low. This requires the use of large sample volumes, which in turn might lead to increased metal ion stripping. Considerable sample pretreatment has been required to overcome these problems, an example being buffer exchange by diafiltration in combination with concentration procedures. Such pretreatment is time consuming, potentially harmful to sensitive proteins, and can cause unnecessary loss of target protein.

His Mag Sepharose excel

His Mag Sepharose excel is a magnetic IMAC medium precharged with nickel ions that are very strongly bound to a chelating ligand. Samples that usually cause stripping of metal ions can therefore be added to the medium with retained protein-binding capacity and without pretreatment. The medium enables an efficient and simplified workflow for capture, purification, and reproducible screening of histidine-tagged proteins expressed and secreted into cell culture supernatants from eukaryotic cells such as insect cells or Chinese Hamster Ovary (CHO) cells.

The Mag Sepharose format has excellent properties for small-scale experiments. The high density of the beads allows rapid capture by magnetic devices while the visibility of the beads ensures reliable collection of the bound histidine-tagged proteins during the purification procedure. The base matrix is based on Sepharose medium with magnetite incorporated. The main characteristics and chemical stability of His Mag Sepharose excel are listed in Table 1 and Table 2 respectively.

His Mag Sepharose excel is available as 10% (v/v) medium slurry in three different pack sizes (2 × 1 ml, 5 × 1 ml, and 10 × 1 ml) and can be used together with different test tubes and magnetic racks, for example MagRack 6 for test tubes up to 1.5 ml or MagRack Maxi for test tubes up to 50 ml. 1 ml medium slurry is sufficient for five reactions according to the recommended protocol. The products are delivered in tubes containing 1 ml of 10% (v/v) medium slurry that contains 100 µl magnetic beads.

Ni Sepharose excel

Ni Sepharose excel is an IMAC medium precharged with nickel ions that are very strongly bound to a chelating ligand. Samples that usually cause stripping of metal ions can therefore be loaded to the medium with retained protein-binding capacity. The nickel ions have been shown to remain bound to the chelating ligand even after incubation for 24 hours in 10 mM EDTA. Ni Sepharose excel is designed primarily for capture and purification of histidine-tagged proteins secreted into cell culture supernatants from eukaryotic cells such as insect cells or CHO cells.

Ni Sepharose excel enables direct loading of samples without having to perform extensive and time-consuming pretreatment procedures. The flow properties of the medium make it excellent for purifications in a wide range of scales and allow loading of large sample volumes, enabling purification of target protein at low concentrations from large volumes.

It is recommended to perform binding at neutral pH. However, successful purification has routinely been observed with binding performed at a pH as low as 6.0.

Ni Sepharose excel can be packed into chromatography columns such as Tricorn™, XK, and HiScale™ columns. The main characteristics and chemical stability of Ni Sepharose excel are listed in Table 1 and Table 2 respectively. Ni Sepharose excel is supplied preswollen in 25, 100, and 500 ml packs as well as prepacked in HisTrap™ excel 1 ml and 5 ml columns.

Table 1. His Mag Sepharose excel and Ni Sepharose excel characteristics

Product	His Mag Sepharose excel	Ni Sepharose excel
Matrix	Highly cross-linked spherical agarose including magnetite	Highly cross-linked spherical agarose
Precharged ion	Nickel	Nickel
Average particle size	63 µm	90 µm
Dynamic binding capacity[1, 2]	At least 10 mg $(histidine)_6$-tagged protein/ml sedimented medium	At least 10 mg $(histidine)_6$-tagged protein/ml sedimented medium
Maximum flow velocity[2,3]	N/A	600 cm/h
pH stability[4]	Working range: 2 to 12 Cleaning-in-place: N/A	Working range: 2 to 12 Cleaning-in-place: 2 to 14
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol

[1] Binding capacity is sample- and protein dependent. The binding capacity for most proteins is considerably higher than 10 mg/ml.
[2] Dynamic binding capacity was tested with 0.5 mg/ml pure $(histidine)_6$-tagged protein (M_r 43 000) or (histidine)6-tagged protein (M_r 28 000) spiked in EX-CELL™ 420 Insect serumfree medium (capacity at 10% breakthrough). Column volume was 1 ml and flow rate 1 ml/min.
[3] H_2O at room temperature.
[4] Optimal flow velocity during binding is sample-dependent. During column wash and elution, a flow velocity of 150 cm/h is recommended.
[5] Working range: pH interval where the medium can be operated without significant change in function. Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

HisTrap excel

HisTrap excel 1 ml and 5 ml are ready-to-use IMAC columns prepacked with Ni Sepharose excel. The design of the columns in combination with the specific properties of the medium enables fast and convenient purifications. The special type of filter in the top and bottom of the columns makes it possible to load large volumes of cell-free, unclarified samples directly on the columns without causing back pressure problems. These time-saving properties help prevent degradation and loss of sensitive target proteins.

HisTrap excel columns are made of biocompatible polypropylene. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet.

HisTrap excel columns can be operated either with a peristaltic pump or with chromatography systems such as ÄKTA™ systems. The columns cannot be opened or refilled. The main characteristics of HisTrap excel 1 ml and 5 ml are listed in Table 3.

Table 2. Chemical stability[1] of His Mag Sepharose excel and Ni Sepharose excel

Substance	Duration of test
0.01 M HCl and 0.01 M NaOH	One week
10 mM EDTA, 1 M NaOH, 5 mM DTT, 5 mM TCEP, 20 mM β-mercaptoethanol and 6 M guanidine-HCl	24 hours
500 mM imidazole and 100 mM EDTA	2 hours
30% 2-propanol	20 minutes

[1] Chemical stability of the product was tested by incubating His Mag Sepharose excel or Ni Sepharose excel (that both have the same ligand) in the listed solutions at room temperature and thereafter measuring either the nickel leakage or the protein binding capacity.

Table 3. Main characteristics of HisTrap excel

Column	HisTrap excel
Medium	Ni Sepharose excel
Column volumes	1 ml and 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml column) 1.6 × 2.5 cm (5 ml column)
Recommended flow rates[1,2]	1 to 4 ml/min (1 ml column) 5 to 20 ml/min (5 ml column)
Maximum flow rates[1]	4 ml/min (1 ml column) 20 ml/min (5 ml column)
Column hardware pressure limit	5 bar (0.5 MPa)

[1] H_2O at room temperature. Maximum flow rate will be lower when using buffers or samples with high viscosity or when performing purification at low temperature.

[2] Optimal flow rate during binding is sample-dependent. During column wash and elution, a flow rate of 1 ml/min and 5 ml/min is recommended for 1 ml and 5 ml columns, respectively.

Note: The maximum pressure the packed bed can withstand depends on the chromatography medium characteristics and sample/liquid viscosity. The value measured on the chromatography system used also depends on the tubing used to connect the column.

Comparison of Ni Sepharose excel and Ni Sepharose 6 Fast Flow

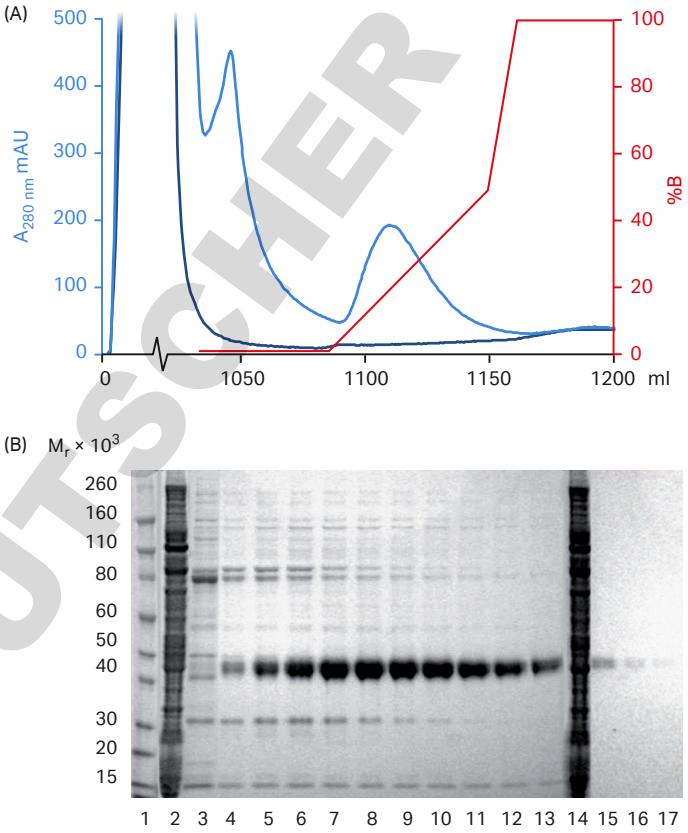
Purification of histidine-tagged protein secreted into CHO cell culture supernatants often causes stripping of the immobilized metal ions on conventional IMAC media such as Ni Sepharose 6 Fast Flow (FF). To investigate the difference in performance between Ni Sepharose excel and Ni Sepharose 6 FF two comparative analyses were performed.

Purification of protein secreted into insect cell culture medium

A histidine-tagged truncated hemagglutinin ($(his)_6$-HA) expressed in High Five™ insect cells and secreted into 1100 ml insect cell culture medium was applied onto HisTrap excel 5 ml and HisTrap FF crude 5 ml columns (prepacked with Ni Sepharose 6 FF).

Figure 4 illustrates the difference in performance between HisTrap excel and HisTrap FF crude. Total protein yield eluted from the HisTrap excel column was estimated to 12.7 mg comprising approximately 8.9 mg of the protein of interest, whereas the amount of protein eluted from HisTrap FF crude was too low to be quantitated. When the flowthrough material from the run on HisTrap FF crude was loaded onto a HisTrap excel 5 ml column, approximately 6 mg of the histidine-tagged protein was successfully recovered. In addition, HisTrap excel retained its blue color after purification whereas HisTrap FF crude did not, further demonstrating that HisTrap excel resists nickel ion stripping. This comparative study clearly demonstrates the advantages of using HisTrap excel when isolating histidine-tagged proteins from a cell culture medium causing metal ion stripping.

Column:	HisTrap excel 5 ml and HisTrap FF crude 5 ml
Sample:	$(his)_6$-HA secreted into GIBCO™ Sf-900 II SFM insect cell culture medium, pH 6.6. No imidazole added.
Sample volumes:	1020 ml and 1012 ml, respectively
Equilibration/wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	5 ml/min
Temperature:	Room temperature
System:	ÄKTAexplorer



1. Novex™ Sharp Pre-stained Protein Standard
2. HisTrap excel, High Five insect cell culture supernatant
3. HisTrap excel, wash
4-13. HisTrap excel, elution fractions 1-10
14. HisTrap crude FF, High Five insect cell culture supernatant
15. HisTrap FF crude, wash
16-17. HisTrap FF crude, elution fractions 1-2

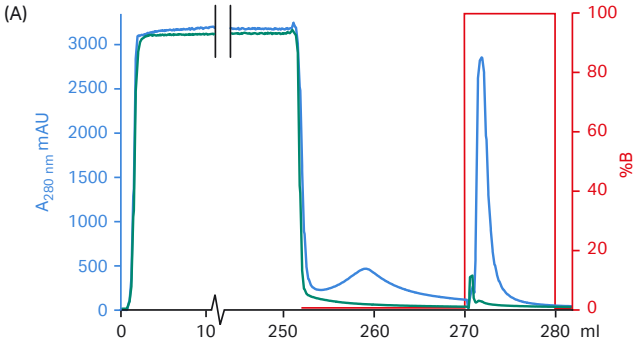
Fig 4. (A) Comparative purification of $(his)_6$-HA in insect cell culture supernatant using HisTrap excel (blue) and HisTrap FF crude (purple); (B) SDS-PAGE analysis of elution fractions from the same purifications. The SDS-PAGE gel (reducing conditions) was stained with SimplyBlue™ SafeStain and analyzed with Gel Doc™ XR+ System.

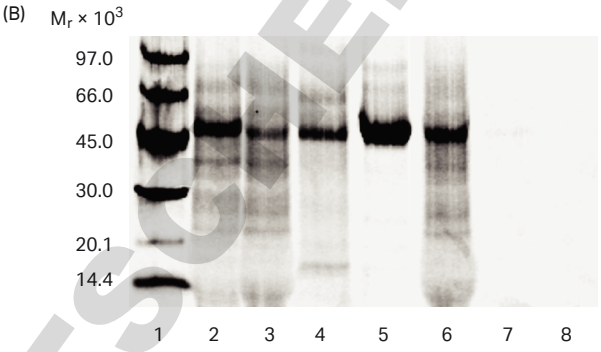
Purification of protein secreted into CHO cell culture medium

To investigate the difference in performance between Ni Sepharose excel and Ni Sepharose 6 FF, a comparative analysis was performed by purification of histidine-tagged murine plasminogen activator inhibitor (mPAI-1-$(his)_8$) expressed in CHO cells and secreted into CHO cell culture medium.

Figure 5 shows that mPAI-1-$(his)_8$ could be successfully purified using Ni Sepharose excel, with a purity of >98%. In comparison, Ni Sepharose 6 FF showed no recovery of target protein. Moreover, Ni Sepharose excel kept its blue color after the experiment while Ni Sepharose 6 FF did not, giving additional evidence that Ni Sepharose excel resists nickel stripping.

Medium:	Ni Sepharose excel, 1 ml	Ni Sepharose 6 FF, 1 ml
Column:	Tricorn 5/50	Tricorn 5/50
Sample:	mPAI-1-$(his)_8$ secreted into GIBCO CD CHO medium, pH 7.0	
Sample volume:	250 ml	250 ml
Equilibration buffer:	20 mM sodium phosphate 500 mM NaCl pH 7.4	20 mM sodium phosphate 500 mM NaCl
Wash buffer:	20 mM sodium phosphate 500 mM NaCl 10 mM imidazole pH 7.4	20 mM imidazole pH 7.4 20 mM sodium phosphate 500 mM NaCl
Elution buffer	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4	20 mM imidazole pH 7.4
Flow rate:	1 ml/min	
Temperature:	Room temperature	
System:	ÄKTA avant 25	





1. LMW-SDS Marker Kit
2. Start material
3. Ni Sepharose excel, flowthrough
4. Ni Sepharose excel, wash
5. Ni Sepharose excel, eluate
6. Ni Sepharose 6 FF, flowthrough
7. Ni Sepharose 6 FF, wash
8. Ni Sepharose 6 FF, eluate

Fig 5. (A) Comparative purification of mPAI-1-$(his)_8$ (M_r 46 300) in CHO cell culture supernatant using Ni Sepharose excel (blue) and Ni Sepharose 6 FF (green); (B) SDS-PAGE analysis. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple™ Total Protein Stain, scanned in Typhoon™ FLA 9000 Imager, and analyzed with ImageQuant™ TL software.

Highly repeatable purification using His Mag Sepharose excel

Using His Mag Sepharose excel magnetic beads, several purifications can be performed simultaneously and with high repeatability. In order to demonstrate this, six purifications of mPAI-1-(his)$_8$ expressed in CHO cells and secreted into CHO cell culture medium were performed using the standard protocol recommended in the product instructions. Figure 6 shows the successful purifications using His Mag Sepharose excel, giving an average purity of 93%.

Both purity and yield were found to be highly repeatable with a relative standard deviation (RSD) of 1.0 and 2.8% respectively.

Medium:	His Mag Sepharose excel, 200 µl 10% slurry
Sample:	mPAI-1-(his)$_8$ secreted into GIBCO CD CHO medium, pH 7.0
Sample volume:	10 ml
Equilibration buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4
Wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

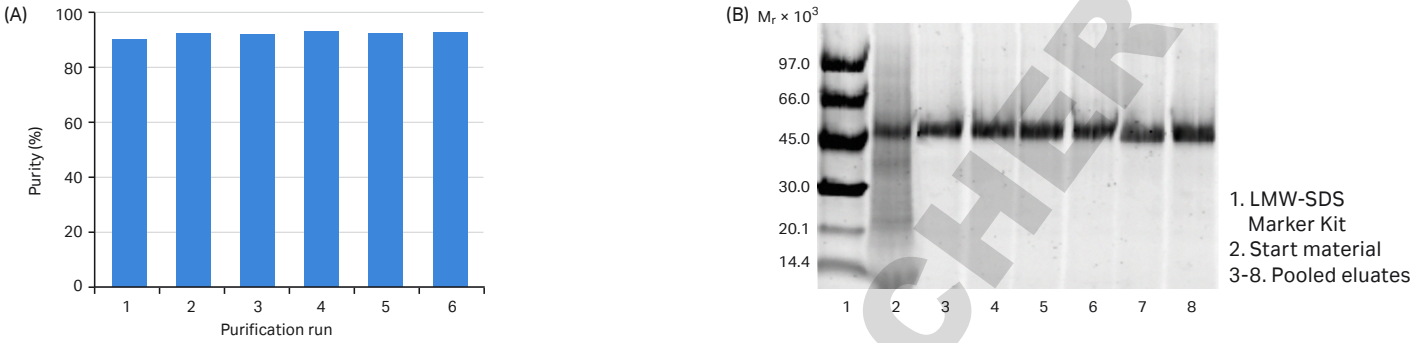
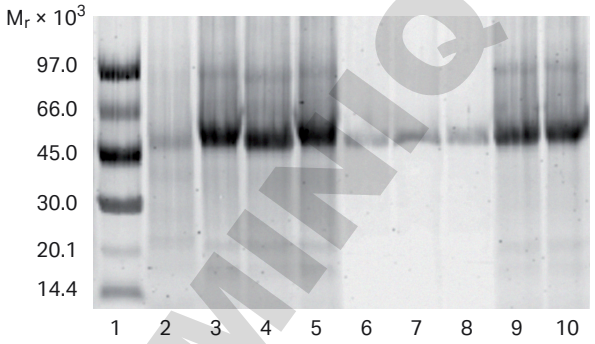


Fig 6. (A) Graph showing purity of six parallel purifications of mPAI-1-(his)$_8$ (M_r 46 300) secreted into CHO cell culture supernatant using His Mag Sepharose excel. (B) SDS-PAGE analysis was used to determine the purity and relative yield of the purifications. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

Comparative study of target protein yield using different magnetic bead media

To investigate the difference in performance between two different magnetic IMAC media, a comparative study was performed at Cytiva laboratories. The protein mPAI-1-(his)$_8$ expressed in CHO cells and secreted into CHO cell culture medium was purified using His Mag Sepharose excel from Cytiva and Ni-NTA Magnetic Agarose Beads from Qiagen according to each manufacturer's protocol, including the recommended two-hour incubation time for Ni-NTA Magnetic Agarose Beads. His Mag Sepharose excel was incubated for 4 hours (the minimum recommended incubation time) and for 16 hours. Sixteen hours was found to be well suited for this protein. Figure 7 shows the SDS-PAGE analysis and results from the study.



1. LMW-SDS Marker Kit
2. Start material
3-5. His Mag Sepharose excel, 16 h, triplicate purifications
6-8. Ni-NTA Agarose Magnetic Beads, 2 h, triplicate purifications
9-10. His Mag Sepharose excel, 4 h, duplicate purifications

Fig 7. SDS-PAGE analysis of pooled elution fractions from purification of mPAI-1-(his)$_8$ (M_r 46 300) secreted into CHO cell culture supernatant, using His Mag Sepharose excel and Ni-NTA Agarose Magnetic Beads. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

Setting the relative yield for His Mag Sepharose excel (16 h) to 100%, the yield obtained with Ni-NTA Magnetic Agarose Beads was 24%. Using Student's t-test, these differences in yield were statistically significant ($p<0.05$, df=4), experiments performed in triplicate. The considerably shorter incubation time (4 h) also resulted in good yield using His Mag Sepharose excel; only 20% less target protein was obtained compared to the 16-hour incubation.

Supplier:	Cytiva	Qiagen
Medium:	His Mag Sepharose excel	Ni-NTA Magnetic Agarose Beads
Medium Volume:	200 µl 10% slurry	400 µl 5% slurry
Sample:	mPAI-1-(his)$_8$ secreted into GIBCO CD CHO medium	
Sample volume:	10 ml	10 ml
Incubation time:	16h or 4h	2h
Equilibration buffer:	20 mM sodium phosphate 500 mM NaCl pH 7.4	50 mM sodium phosphate 300 mM NaCl 10 mM imidazole 0.05% Tween™ 20 pH 8.0
Binding conditions:	No additions to the sample, pH adjusted to pH 7.5	50 mM sodium phosphate 300 mM NaCl 10 mM imidazole pH 8.0
Wash buffer:	20 mM sodium phosphate 500 mM NaCl 10 mM imidazole pH 7.4	50 mM sodium phosphate, 300 mM NaCl, 20 mM imidazole 0.05% Tween 20 pH 8.0
Elution buffer	20 mM sodium phosphate 500 mM NaCl 500 mM imidazole pH 7.4	50 mM sodium phosphate 300 mM NaCl 500 mM imidazole 0.05% Tween 20 pH 8.0

Convenient screening and scale-up

Purification of histidine-tagged proteins by IMAC is a choice between yield and purity. Low concentration of imidazole in the wash buffer promotes high yield while higher imidazole concentrations increase purity. The optimal imidazole concentration depends on the protein and sample properties. A screening experiment can be done to investigate the effects of imidazole.

One of the key advantages of magnetic bead purification is the possibility to vary the volumes of sample and medium slurry. This property makes His Mag Sepharose excel highly suitable for screening experiments, providing a seamless transfer of the chosen purification conditions to larger scales.

Screening for optimal imidazole concentration

Using His Mag Sepharose excel, a study was undertaken to investigate the effects of wash buffer imidazole concentration on purity and yield during the purification of histidine-tagged prolylcarboxypeptidase (PRCP-$(his)_9$) expressed in High Five insect cells and secreted into insect cell culture medium. Figure 8 shows the SDS-PAGE analysis of wash and eluted pools (triplicate purifications). A good balance between purity and recovery was achieved with 30 mM imidazole, and this concentration was chosen for the subsequent scale-up study.

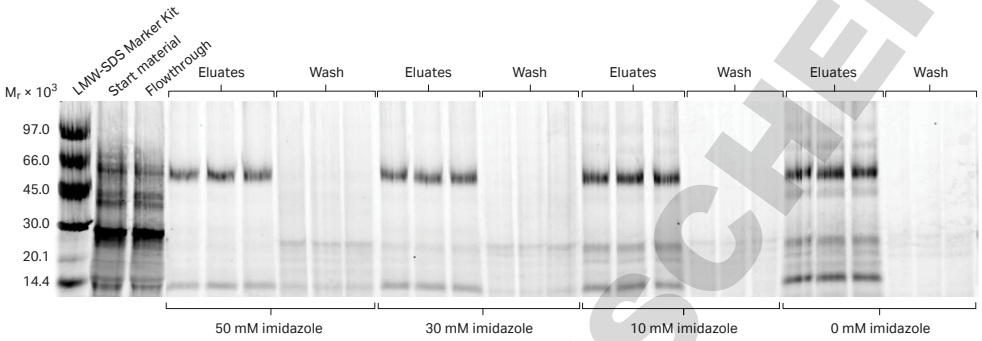


Fig 8. SDS-PAGE analysis of eluted pools from purification of PRCP-$(his)_9$ (M_r 54 600) secreted into insect cell culture supernatant using His Mag Sepharose excel. Purification was performed in triplicate using 0-50 mM imidazole in wash buffers. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain. Running conditions can be found in Table 4.

Scale-up using His Mag Sepharose excel and HisTrap excel

The flexibility of His Mag Sepharose was investigated by a 5-fold purification scale-up of PRCP-$(his)_9$ using 30 mM imidazole in the wash buffer. Figure 9A shows the eluted pools from purifications using different medium slurry volumes, with recovery and purity found to differ less than 10% between small and large scale.

The information obtained from screening using His Mag Sepharose excel can also be used for scale-up to other formats. In order to demonstrate this, a 50-fold purification scale-up was performed comparing 200 µl His Mag Sepharose excel 10% medium slurry with column purification on HisTrap excel 1 ml (step elution). The results in Figure 9B demonstrate comparable purity and recovery between the formats, thus confirming the reliability of purification scale-up from magnetic beads to column format.

Table 4. Experimental conditions for scale-up using His Mag Sepharose excel and HisTrap excel

Medium /Column:	His Mag Sepharose excel	His Mag Sepharose excel	HisTrap excel
Medium slurry/bed volume:	200 µl 10% medium slurry	1 ml 10% medium slurry	1 ml
Sample volume:	10 ml	50 ml	500 ml
Sample:	PRCP-$(his)_9$ secreted into SAFC EX-CELL 405 medium, pH 6.9		
Equilibration buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4		
Wash buffer:	20 mM sodium phosphate 0-50 mM imidazole 500 mM NaCl pH 7.4	20 mM sodium phosphate 30 mM imidazole 500 mM NaCl pH 7.4	20 mM sodium phosphate 30 mM imidazole 500 mM NaCl pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4		
Binding/ wash flow rate:	-	-	2 ml/min
Elution flow rate:	-	-	1 ml/min
System:	-	-	ÄKTA avant 25

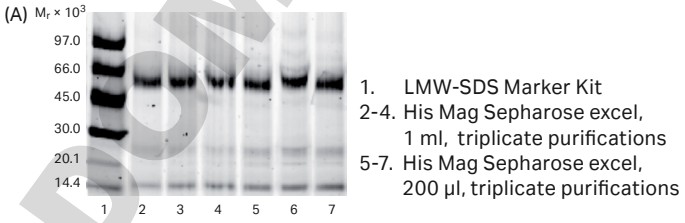


Fig 9. SDS-PAGE analysis of eluted pools from purification of PRCP-$(his)_9$ (M_r 54 600) secreted into insect cell culture supernatant with scale-up from (A) 200 µl His Mag Sepharose excel slurry to 1 ml His Mag Sepharose excel slurry; (B) 200 µl His Mag Sepharose excel slurry to HisTrap excel 1 ml. Purifications using His Mag Sepharose excel were performed in triplicate. The SDS-PAGE gels (reducing conditions) were stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software. Running conditions can be found in Table 4.

Functional compatibility of Ni Sepharose excel with EDTA

Recombinantly expressed proteins secreted into the cell culture medium yield low protein concentration in large volumes, leading to extended loading times during chromatography purification. Inhibitors are therefore frequently used after harvest to avoid degradation of the target protein. Ethylenediaminetetraacetic acid (EDTA) is often used as a metalloprotease inhibitor, alone or as a component in protease inhibitor cocktails.

Conventional IMAC media are very sensitive to EDTA because it causes stripping of the immobilized metal ions. Ni Sepharose excel has been developed to resist metal ion stripping. A purification study was undertaken in order to test the behavior of Ni Sepharose excel with EDTA added to the samples. Histidine-tagged procarboxy peptidase U (proCPU-$(his)_8$) was expressed in Sf9 insect cells and secreted into insect cell culture medium. Supernatants supplemented with 0, 2, or 10 mM EDTA were run on separate HisTrap excel columns. The overlaid chromatograms in Figure 10A display relatively good conformity between runs with a tendency towards an increased amount of eluted material with higher EDTA concentration. SDS-PAGE analysis of the eluates from the three runs revealed an average purity for proCPU-$(his)_8$ of 74%. These results show that Ni Sepharose excel medium is functionally compatible with EDTA concentrations of at least 10 mM, a concentration that exceeds the amount of EDTA in most enzyme inhibitor cocktails.

Column:	HisTrap excel 1 ml
Sample:	proCPU-$(his)_8$ secreted into GIBCO Sf-900 II insect cell medium, pH 6.8, later supplemented with 0 mM, 2 mM, or 10 mM EDTA
Sample volume:	250 ml
Equilibration buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4
Wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 15 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Binding/wash flow rate:	2 ml/min
Elution flow rate:	1 ml/min
Temperature:	Room temperature
System:	ÄKTA avant 25

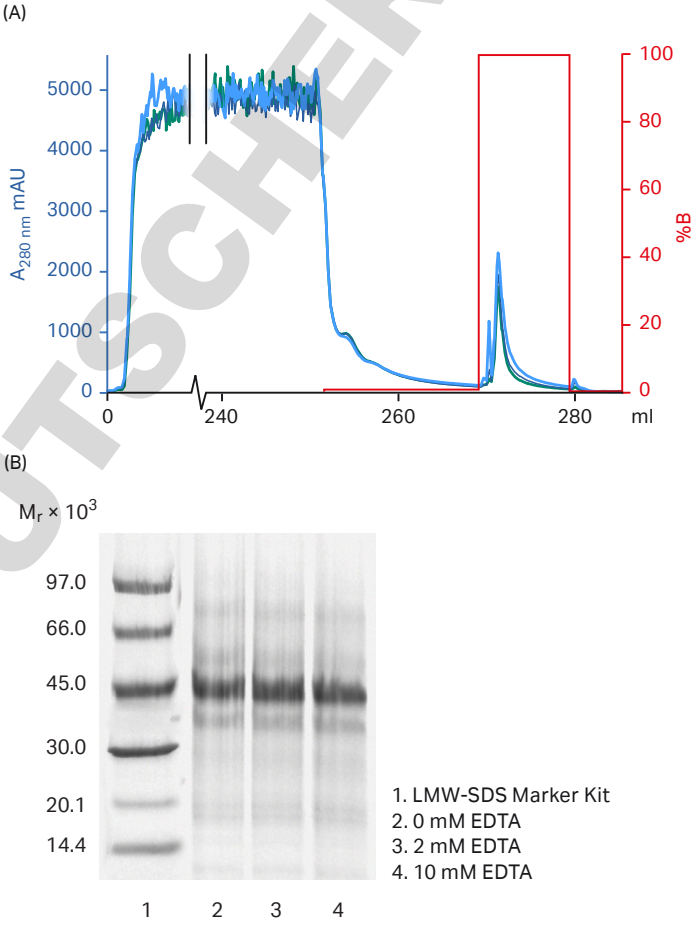


Fig 10. (A) Chromatogram showing the purification of proCPU-$(his)_8$ (M_r 48 300) secreted into insect cell supernatant supplemented with 0 mM, 2 mM, and 10 mM EDTA using HisTrap excel 1 ml columns. The small peaks in front of the main elution peaks are most likely a consequence of aggregation during storage of the sample at room temperature for an extended period of time during the runs; (B) SDS-PAGE analysis of elution pools. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain, scanned in Typhoon FLA 9000 Imager, and analyzed with ImageQuant TL software.

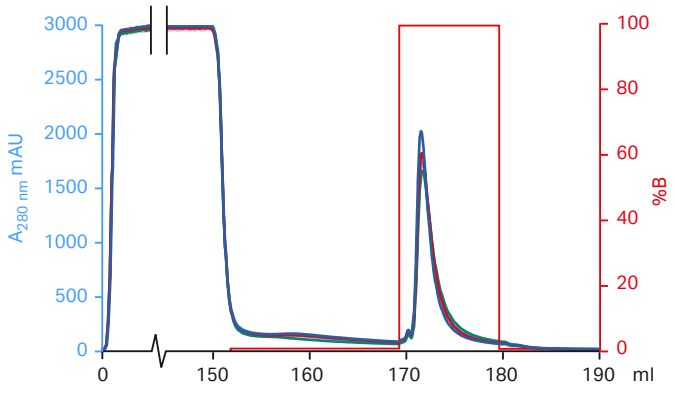
Reusability of a HisTrap excel column

In order to test the reusability of HisTrap excel columns, a study was undertaken where five consecutive runs on the same column were performed without cleaning or regeneration between the runs. 200 ml cell culture supernatant with mPAI-1-$(his)_8$ expressed in CHO cells was loaded onto the 1 ml column in each run. Figure 11A shows an overlay of chromatograms from the five runs, demonstrating very good reproducibility between runs (RSD for integrated curve area: 2.7%).

Figure 11B further demonstrates the excellent reusability properties of HisTrap excel with retained purification functionality over the five runs.

Column:	HisTrap excel 1 ml
Sample:	mPAI-1-$(his)_8$ secreted into GIBCO CD CHO medium, pH 7.0
Sample volume:	200 ml
Equilibration buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4
Wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Binding/ wash flow rate:	2 ml/min
Elution flow rate:	1 ml/min
Temperature:	4ºC
System:	ÄKTA avant 25

(A)



(B)

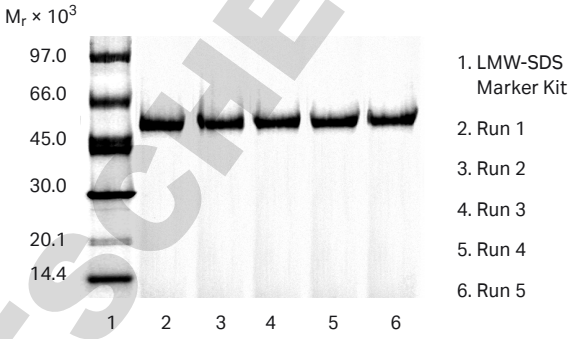
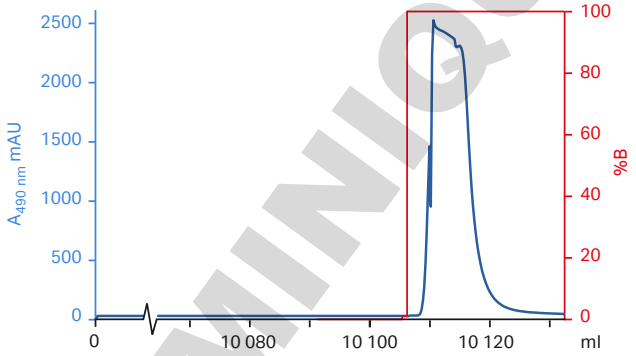


Fig 11. (A) Overlaid chromatograms from five consecutive purifications of mPAI-1-$(his)_8$ (M_r 46 300) secreted into CHO cell culture supernatant on a single HisTrap excel 1 ml column; (B) SDS-PAGE analysis of eluted pools from the purification runs. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain, scanned in Typhoon FLA 9000 Imager, and analyzed with ImageQuant TL software.

Rapid large-volume purification on HisTrap excel

The possibility to load large sample volumes on HisTrap excel 5 ml was investigated by application of 10 liters of CHO cell culture medium containing green fluorescent protein (GFP-$(his)_6$) added to a concentration of 5 mg/l. Figure 12 shows that the large sample volume could be successfully processed using a flow rate as high as 20 ml/min. The recovery determined by absorbance measurements at 490 nm was 89%.



Column:	HisTrap excel 5 mll
Sample:	5 mg/l GFP-$(his)_6$ added to PowerCHO™ 2 culture medium with 2 mM L-glutamine, pH 6.3
Sample volume:	10 l
Equilibration buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4
Wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 15 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Binding/wash flow rate:	20 ml/min
Elution flow rate:	10 ml/min
Temperature:	Room temperature
System:	ÄKTA avant 25

Fig 12. Purification of 10 l 5 mg/l GFP-$(his)_6$ in CHO cell culture medium using HisTrap excel 5 ml. The extra peak seen at the start of elution was caused by a system pause initiated because the high pressure limit had been reached by an initial elution flow rate of 20 ml/min. Elution was continued at 10 ml/min.

Ni Sepharose excel: Impact of imidazole on purity and yield when purifying proteins secreted into insect cell culture media

Imidazole is commonly used in wash and elution buffers when purifying histidine-tagged proteins on IMAC columns. Varying the imidazole concentration in the wash buffer influences both purity and yield of the target protein. Purification of histidine-tagged proteins secreted into insect cell culture media often requires optimization of the imidazole wash concentration. Purity and yield were therefore studied using 10, 30, or 50 mM imidazole during wash in three separate runs. PRCP-$(his)_9$ expressed in High Five insect cells and secreted into insect cell culture medium was purified using HisTrap excel 1 ml. Figure 13 and Figure 14 clearly demonstrate how the concentration of imidazole in the wash buffer influences the relationship between protein purity and yield, with higher imidazole concentration giving higher purity but lower yield. Thus the appropriate imidazole concentration depends on whether it(is yield or purity that is most important for the task at hand.

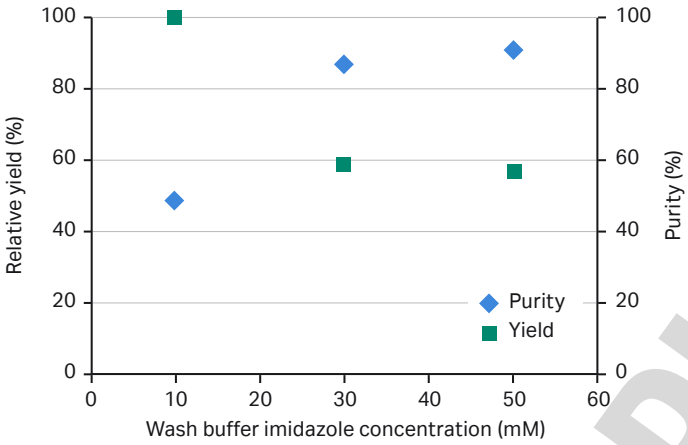


Fig 13. Protein purity and relative yield calculated from the SDS-PAGE analysis (Fig 14) and plotted against wash buffer imidazole concentration.

Column:	HisTrap excel 1 ml
Sample:	PRCP-$(his)_9$ secreted into SAFC EX-CELL 405 medium, pH 6.9
Sample volume:	500 ml
Equilibration buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4
Wash buffer:	20 mM sodium phosphate, 500 mM NaCl, 10, 30, or 50 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Binding/wash flow rate:	2 ml/min
Elution flow rate:	1 ml/min
Temperature:	Room temperature
System:	ÄKTA avant 25

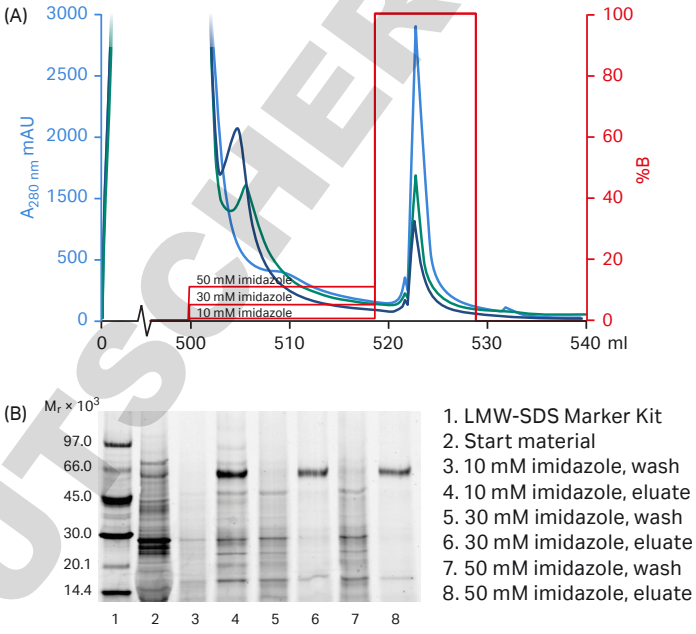


Fig 14. (A) Overlaid chromatograms from three separate purifications of PRCP-$(his)_9$ secreted into insect cell culture supernatant with varying imidazole concentration in the wash buffer: 10 mM (blue), 30 mM (green), and 50 mM (purple); (B) SDS-PAGE analysis. The SDS-PAGE gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

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The clone with GFP-$(his)_6$ was kindly provided by Drs. Jan-Willem de Gier and David Drew, Dept. of Biochemistry and Biophysics, Stockholm University, Sweden.

Ordering information

Product	Quantity	Code number
Ni Sepharose excel	25 ml	17-3712-01
Ni Sepharose excel	100 ml	17-3712-02
Ni Sepharose excel	500 ml	17-3712-03
HisTrap excel	1 × 1 ml	29-0485-86
HisTrap excel	5 × 1 ml	17-3712-05
HisTrap excel	5 × 5 ml	17-3712-06
His Mag Sepharose excel	2 × 1 ml	17-3712-20
His Mag Sepharose excel	5 × 1 ml	17-3712-21
His Mag Sepharose excel	10 × 1 ml	17-3712-22
MagRack 6	1	28-9489-64
MagRack Maxi	1	28-9864-41

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
Total solutions for histidine-tagged proteins, Selection Guide	28-4070-92
Protein Sample Preparation Handbook	28-9887-41

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