His MultiTrap FF and His MultiTrap HP

His MultiTrap™ FF and His MultiTrap HP are prepacked, 96-well filter plates for screening and small-scale, high-throughput parallel purification of histidine-tagged recombinant proteins by immobilized metal ion affinity chromatography (IMAC). Consistent well-to-well and plate-to-plate performance ensures high reproducibility. The ability to load unclarified lysates on His MultiTrap plates simplifies the workflow and saves time. The standardized 96-well plate format gives great flexibility, both when working with automated robotic systems and when manually using centrifugation or vacuum. Using the filter plates, 96 samples with up to 1 mg of histidine-tagged protein/well can be purified in 60 min.

Fig 1. His MultiTrap FF and His MultiTrap HP prepacked 96-well plates allow fast screening and small-scale, high-throughput parallel purifications.

Key performance benefits include:

- Highly reproducible well-to-well and plate-to-plate results.
- Less sample pretreatment needed – load unclarified sample directly, increase reproducibility in results, and save time.
- Easy and predictable scale-up to HisTrap™ FF, HisPrep™ FF 16/10, or HisTrap HP prepacked columns.
- High chemical stability and high binding capacity– purifies up to 1 mg of histidine-tagged protein per well.
- Prepacked with Ni Sepharose™ media, which have low nickel leakage and are compatible with a wide range of additives used in protein purification.

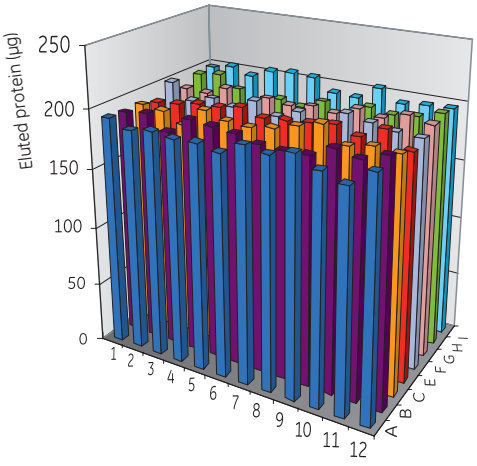

Fig 2. High well-to-well reproducibility when screening with His MultiTrap HP plates.

Product characteristics

96-well plates

The 96-well plate (Fig 1) is made of bio-inert polypropylene and polyethylene. Each well has a volume of 800 µl, and each is prepacked with 50-µl medium in 20% ethanol (500 µl solution of 10% slurry). His MultiTrap FF and His MultiTrap HP prepacked 96-well plates give simple, yet highly reproducible purifications of up to 1 mg histidine-tagged protein/well from unclarified samples. Figure 2 shows the high well-to-well reproducibility that is achieved in the purification using His MultiTrap 96-well plates.

Plates are supplied in packs of four and are sealed at the top and bottom on delivery.

Prepacked media

His MultiTrap FF and His MultiTrap HP are prepacked with Ni Sepharose 6 Fast Flow (90-µm beads of highly cross-linked agarose) and Ni Sepharose High Performance (34-µm beads), respectively (Table 1).

Table 1. His MultiTrap FF and His MultiTrap HP characteristics

Filter plate size[1]	127.8 × 85.5 × 30.6 mm, according to ANSI/SBS 1-2004, 3-2004 & 4-2004 standards
Filter plate material	Polypropylene and polyethylene
Media	
His MultiTrap FF	Ni Sepharose 6 Fast Flow, (precharged with Ni^{2+})
His MultiTrap HP	Ni Sepharose High Performance, (precharged with Ni^{2+})
Average bead size	
Ni Sepharose 6 Fast Flow	90 µm
Ni Sepharose High Performance	34 µm
Metal ion capacity	~15 µmol Ni^{2+}/ml medium
Binding capacity[2, 3]	
His MultiTrap FF	Up to 0.8 mg histidine-tagged protein/well
His MultiTrap HP	Up to 1 mg histidine-tagged protein/well
Reproducibility between wells[4]	± 10%
Medium volume/well	50 µl (total vol. 500 µl of 10% slurry)
Well volume	800 µl
Number of wells	96
Maximum sample volume/well	600 µl
Centrifugation speed[5]	
Recommended	100–500 × g
Maximum	700 × g
Vacuum pressure[5]	
Recommended	-0.1 to -0.3 bar
Maximum	-0.5 bar
Compatibility with additives	Stable in all commonly used buffers, reducing agents, denaturants, and detergents, see Table 2
Chemical stability[6]	0.01 M HCl, 0.1 M NaOH (tested for one week at 40°C)
	1 M NaOH or 70% acetic acid (tested for 12 h)
	2% SDS (tested for 1 h)
	30% 2-propanol (tested for 30 min)
Avoid in buffers	Chelating agents, e.g. EDTA, EGTA, citrate, see Table 2
pH stability[6]	*Short-term (2 h):* 2–14 *Long-term (one week):* 3–12
Storage solution	20% ethanol
Storage temperature	4°C to 30°C

1 According to ANSI/SBS 1-2004, 3-2004 & 4-2004 standards. ANSI = American National Standards Institute, SBS = Society for Biomolecular Screening.

2 Optimum yield is obtained with protein loads of up to 0.4 and 0.5 mg protein per well using His MultiTrap FF and His MultiTrap HP respectively. Yield can be increased by decreasing the imidazole concentration at binding/wash, but this is at the expense of purity.

3 Binding capacity is protein-dependent.

4 The amount of eluted target protein/well does not differ more than ± 10% from the average amount/well for the whole plate.

5 Depends on sample pretreatment and sample properties.

6 Ni^{2+}-stripped media.

A chelating ligand has been immobilized to both media at a density that ensures a high binding capacity for histidine-tagged proteins when charged with Ni^{2+} ions.

Table 1 lists His MultiTrap FF and His MultiTrap HP characteristics in more detail. Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance media are also compatible with compounds commonly used when purifying histidine-tagged proteins (Table 2). Purifications can be performed either under native or denaturing conditions, and a wide range of detergents, reducing agents, and other additives can be used. For example, both media are stable in DTT and DTE at concentrations up to at least 5 mM. Furthermore, leakage of Ni^{2+} ions is negligible under all normal conditions.

Table 2. Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance are compatible with the following compounds at least at the concentrations stated

Compound	Concentration
Reducing agents[1]	5 mM DTE 5 mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione
Denaturing agents	8 M urea[2] 6 M Guanidine-HCl[2]
Detergents	2% Triton™ X-100 (nonionic) 2% Tween™ 20 (nonionic) 2% NP-40 (nonionic) 2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	500 mM imidazole 20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA[3] 60 mM citrate[3]
Buffers	50 mM sodium phosphate, pH 7.4 100 mM Tris-HCl, pH 7.4 100 mM Tris-acetate, pH 7.4 100 mM HEPES, pH 7.4 100 mM MOPS, pH 7.4 100 mM sodium acetate, pH 4.0[2]

1 For best results, perform a blank run before including reducing agents in the sample/buffers. See Instructions 11-0036-62.

2 Tested for one week at 40°C.

3 The strong chelator EDTA has been used successfully in some cases at 1 mM. In general, use chelating agents with caution (and only in the sample, not in the buffers). Metal-ion stripping may be counteracted by adding a small excess of $MgCl_2$ before sample centrifugation/filtration. Effects may vary with the applied sample volume.

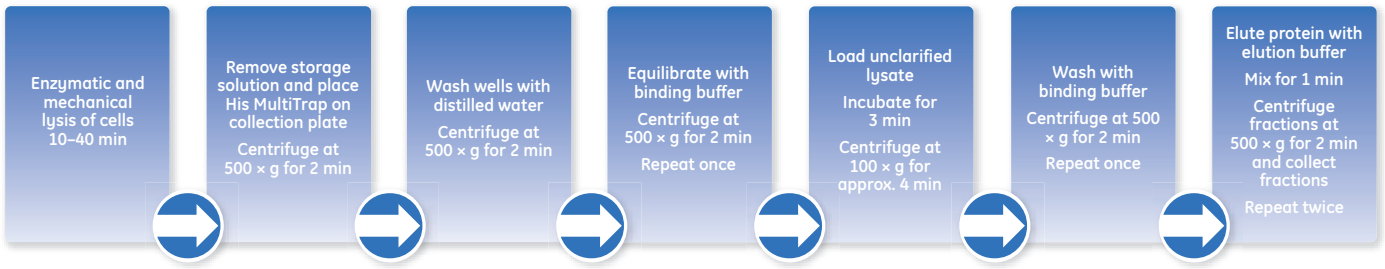

Fig 3. Protocol for purifying histidine-tagged proteins by centrifugation using His MultiTrap 96-well plates. A typical purification can be achieved in approximately 60 min.

Operation

Both plates can be used with a robotic system or operated manually by centrifugation or vacuum. Both confer many operational advantages for screening and parallel purifications of histidine-tagged proteins. Prepacked plates give high consistency and save time. Loading unclarified sample simplifies the workflow for screening as less time is spent on sample preparation.

Simple sample preparation

His MultiTrap FF and His MultiTrap HP purify histidine-tagged proteins directly from unclarified cell lysates. No centrifugation or filtration is needed prior to loading the wells. Samples are prepared by straightforward chemical and/or mechanical lysis. If the sample is viscous, simply extend the mechanical treatment.

A general sample preparation protocol involves: (1) suspending the cells/cell paste, (2) enzymatic lysis using lysozyme, DNAse I, and adding $MgCl_2$ etc, (3) mechanical lysis by sonication, homogenization, or freeze/thaw, (4) adjusting pH, and (5) applying unclarified lysate directly to the wells.

The procedure shown in Figure 3 describes a centrifugation-based protein purification protocol[1].

[1] Refer to His MultiTrap FF and His MultiTrap HP Instructions 11-0036-62 for the full protocol.

Easy scale-up with reliable results

Scaling up from His MultiTrap FF and His MultiTrap HP to columns prepacked with the same medium is simple and reliable (see Applications). Both Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance are available in larger prepacked formats as HisTrap 1-ml and 5-ml columns for syringe, peristaltic pump, or chromatography system operation. Ni Sepharose 6 Fast Flow is also available in His GraviTrap™ 1-ml gravity-flow columns, in HisTrap FF crude 1-ml and 5-ml columns, as well as HisPrep FF 16/10 columns (see Ordering information). As the same conditions (e.g. buffers, imidazole concentrations, gradients, etc.) apply to the His MultiTrap, HisTrap, His Prep, and His GraviTrap formats, scaled-up purification results are highly consistent and optimization times short.

Applications

Several applications have been performed with both His MultiTrap FF and His MultiTrap HP at GE Healthcare and at the Karolinska Institutet, Stockholm, Sweden.

1. High reproducibility of histidine-tagged protein purification from unclarified and clarified lysates using His MultiTrap

Histidine-tagged maltose binding protein (MBP-$[His]_6$) from both clarified and unclarifed *E. coli* lysates was purified on His MultiTrap FF and His MultiTrap HP. Purity, capacity, and reproducibility were analyzed. Table 3 describes the method used. Table 4 and Figure 4 show the results. The unclarified samples drained easily from the wells of both plates and no difference from the way clarified samples drained was observed.

Table 3. Method used for the purification of MBP-$(His)_6$ from clarified and unclarified *E. coli* lysate on His MultiTrap 96-well plates

96-well plate	A) His MultiTrap FF B) His MultiTrap HP
Sample	250 µg/well histidine-tagged maltose binding protein (MBP-$[His]_6$) in clarified or unclarified *E. coli* lysate
Sample preparation	Chemical lysis and sonication
Sample volume	250 µl
Elution volume	3 × 200 µl
Elution method	Centrifugation
Binding buffer	A) 20 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, pH 7.4 B) 20 mM sodium phosphate, 500 mM NaCl, 35 mM imidazole, pH 7.4
Elution buffer	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

Table 4. Purification data for His MultiTrap FF and His MultiTrap HP loaded with clarified and unclarified samples

Characteristic	His MultiTrap FF clarified sample	His MultiTrap FF unclarified sample	His MultiTrap HP clarified sample	His MultiTrap HP unclarified sample
Average amount of eluted protein[1]	192 µg	194 µg	203 µg	228 µg
Relative standard deviation (%)	3%	2%	5%	3%
Recovery	77%	78%	81%	91%

[1] 250 µg/well MBP-$(His)_6$ was loaded to each well. Eluted protein was measured at a UV absorbance of 280 nm. As small amounts of UV-absorbing contaminants are present in the eluted fractions, the values given are only estimates.

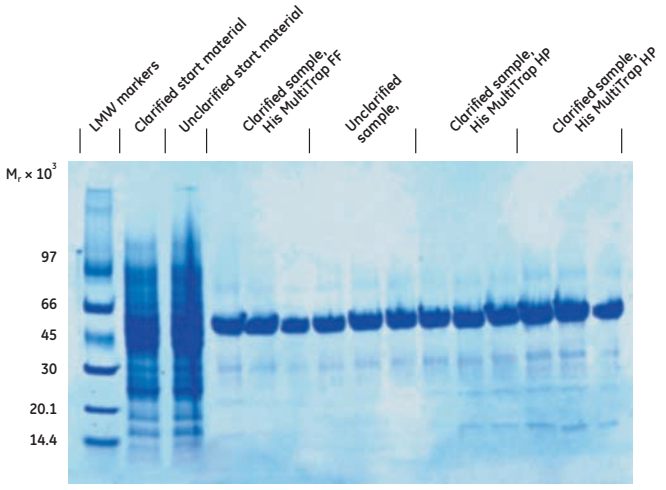

Fig 4. SDS-PAGE (ExcelGel™ SDS Gradient 8–18) of the purification of MBP-$(His)_6$ from clarified and unclarified *E. coli* lysate on His MultiTrap FF and His MultiTrap HP. Elutions 1–3 are shown from each purification.

Table 4 shows that purifying histidine-tagged protein from unclarified samples gives a somewhat higher recovery on His MultiTrap HP (probably due to avoiding sample losses caused when filtering the sample to clarify it), but that no significant difference was seen using His MultiTrap FF. SDS-PAGE showed similar purity of the eluted MBP-$(His)_6$ between clarified and unclarified samples (Fig 4).

The high reproducibility of the MultiTrap purification format is shown in Figure 2 on page 1 of this data file.

2. *Solubility effects of detergents in buffers during purification of membrane proteins*

Eight detergents were screened for their effect on the solubility of six histidine-tagged membrane proteins (EM01, EM04, EM05, EM08, EM29, and EM43). Purifications were run on His MultiTrap FF and His MultiTrap HP. The method used for the screening is described in Table 5.

Figure 5 shows nitrocellulose membrane dot-blots of the fractions collected from the purification of EM05 and EM08 in the presence of different detergents on His MultiTrap FF and His MultiTrap HP. Figure 6 shows the corresponding results for EM29 and EM43.

Table 5. Method used for determining solubility effects of different detergents on membrane proteins using His MultiTrap FF and His MultiTrap HP 96-well plates

96-well plate	A) His MultiTrap FF B) His MultiTrap HP
Sample	Six *E. coli* lysates containing histidine-tagged membrane proteins EM01, EM04, EM05, EM08, EM29, or EM43 EM01 = probable transporter EM04 = ion transporter EM05 = putative transferase EM08 = regulatory protein EM29 = GlpG protein EM43 = cation transporter
Sample preparation	Chemical and freeze/thaw lysis
Sample volume	100 µl
Elution method	Centrifugation
Elution volume	3 × 50 µl
Lysis buffer	20 mM sodium phosphate, 100 mM sodium chloride, 20 mM imidazole, 0.5 mM TCEP, 5 u/ml benzonase, 1 mg/ml lysozyme, EDTA-free protease inhibitor cocktail, 1% to 2% detergent, pH 7.4
Binding buffer	20 mM sodium phosphate, 500 mM sodium chloride, 20 mM imidazole, 0.5 mM TCEP, 1% to 2% detergent, pH 7.4
Wash buffer[1]	20 mM sodium phosphate, 500 mM sodium chloride, 40 mM imidazole, 0.5 mM TCEP, 0.03% dodecyl maltoside (DDM), 1% to 2% detergent, pH 7.4
Elution buffer[1]	20 mM sodium phosphate, 500 mM sodium chloride, 500 mM imidazole, 0.5 mM TCEP, 0.03% dodecyl maltoside (DDM), 1% to 2% detergent, pH 7.4
Detergents	1% Fos-Choline 12 (FC12), 1% undecyl maltoside (UDM), 1% dodecyl maltoside (DDM), 1% Cymal™ 5, 1% Cymal 6, 2% octyl glucoside (OG), 1% Triton™ X-100 (TX-100), 1% lauryl dimethylamine oxide (LDAO)
Data evaluation	Dot-blot analysis on nitrocellulose membrane Histidine-tagged proteins were detected using INDIA HisProbe™ – HRP Western blotting probe SDS-PAGE with Coomassie staining

[1] The same imidazole concentrations were used for binding, wash, and elution of protein, respectively, on both His MultiTrap HP (containing Ni Sepharose High Performance) and His MultiTrap FF (containing Ni Sepharose 6 Fast Flow) plates. Ni Sepharose High Performance has a higher binding capacity and normally needs a slightly higher concentration of imidazole for binding and wash than Ni Sepharose 6 Fast Flow to achieve similar purity.

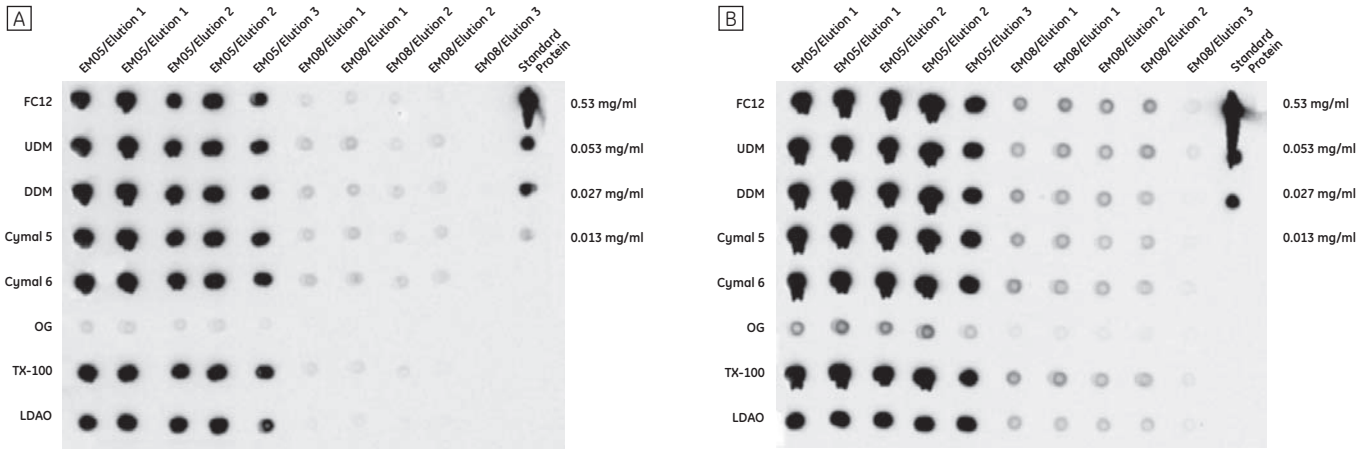

Fig 5. Dot-blots of (A) Membrane proteins EM05 and EM08 purified on His MultiTrap FF, and (B) EM05 and EM08 purified on His MultiTrap HP. Repeats of eluates 1 and 2 shown in the dot-blots are two independent extractions and purifications.

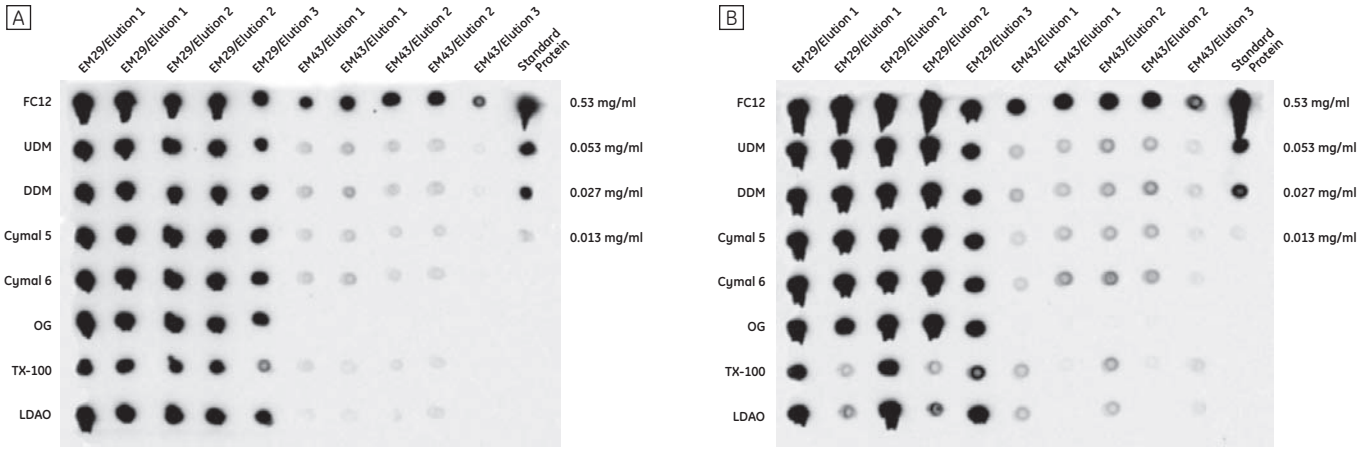

Fig 6. Dot-blots of (A) Membrane proteins EM29 and EM43 purified on His MultiTrap FF, and (B) EM29 and EM43 purified on His MultiTrap HP in the presence of different detergents. Repeats of eluates 1 and 2 shown in the dot-blots are two independent extractions and purifications.

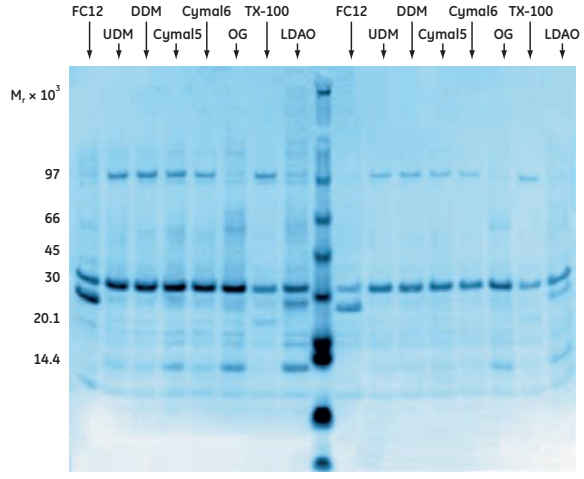

Fig 7. SDS PAGE (Coomassie staining) of EM29 purifications (elutions 1 and 2 in Fig 5) with eight different detergents on His MultiTrap FF.

Both sets of results confirm the high reproducibility between wells and demonstrate the performance of His MultiTrap 96-well plates in the presence of detergents.

In general, Ni Sepharose High Performance (i.e. His MultiTrap HP) has a slightly higher binding capacity than Ni Sepharose 6 Fast Flow (His MultiTrap FF). This is most evident in dots with low signals, for example detergent OG in Figures 5 and 6 and protein EM08 in Figure 5. The difference in capacity might not always be relevant for high expression proteins, but it will be noticeable for those with lower levels of expression such as EM08 and EM43.

SDS-PAGE (Coomassie™ staining) was performed on the 16 fractions (elution steps 1 and 2 from the EM29 purification) with eight different detergents.

3. Scaling up purification of a membrane protein with HisTrap FF crude column using ÄKTAexplorer

When scaling up purification from His MultiTrap plates to larger formats, it is advantageous to use the same medium in the whole process (in each step of the scale-up). The optimized method from screening can be used directly. Purification of EM05, the membrane protein used in the detergent screening study, was scaled up 25-fold with HisTrap FF crude column. Further polishing of the protein was performed by gel filtration using a HiLoad™ 16/60 Superdex™ 200 pg column.

Table 6. Method used for scaling up purification of a histidine-tagged membrane protein from a His MultiTrap FF plate to HisTrap FF crude 1-ml column

Columns	(A) HiTrap FF crude, 1 ml (B) HiLoad 16/60 Superdex 200 pg
Sample	*E. coli* lysate containing histidine-tagged membrane protein (putative transferase EM05)
Sample preparation	Chemical lysis and freeze/thaw
Sample volume	5 ml loaded on HisTrap FF crude column
Lysis, binding, wash, and elution buffers	See Table 5 (detergent FC12 [1%] was used)
Gel filtration buffer	20 mM Tris-HCl, 50 mM sodium chloride, 0.5 mM TCEP, 0.03% DDM, pH 8.0
System	ÄKTAexplorer™
Analysis	UV-absorbance and SDS-PAGE (Coomassie staining)

The same conditions used to purify histidine-tagged EM05 on the His MultiTrap FF plate (Table 5) were used for the HisTrap FF crude 1-ml column, but imidazole gradient elution was preferred. Figure 8 shows the elution profile from HisTrap FF crude, the eluted EM05 fraction run on HiLoad 16/60 Superdex 200 pg, and SDS-PAGE of the purified fraction. As the SDS-PAGE shows in Figure 8, the scale-up resulted in a highly pure protein.

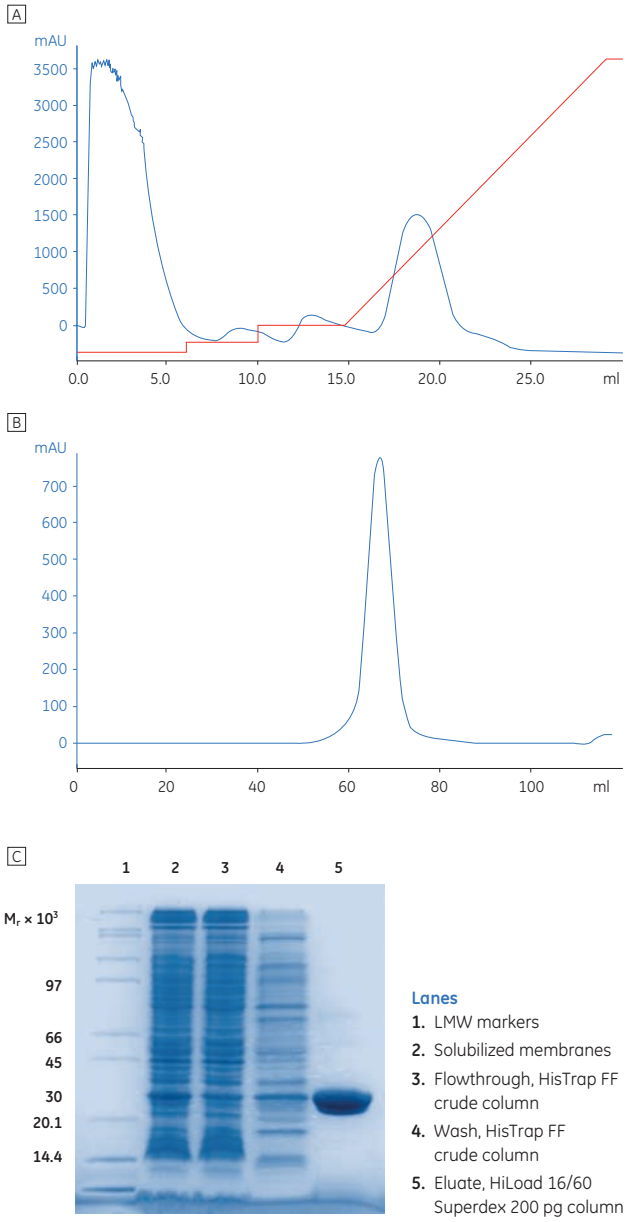

Fig 8. Purification of membrane protein EM05 scaled up from His MultiTrap FF to HisTrap FF crude 1-ml column (A) and further polished on a HiLoad 16/60 Superdex 200 pg column (B). SDS-PAGE with Coomassie staining (C) reveals a highly pure protein.

4. *Screening the expression levels of soluble proteins in eight replicates*

In this study, 12 soluble histidine-tagged proteins (AH03, AH04, AH06, AH09, AH13, AH15, AH17, AH18, AH34, AH53, AH64, and AH65), all expressed in *E. coli,* were purified on His MultiTrap FF and His MultiTrap HP. Eight replicates were run. The purification protocol is essentially the same as in Table 5, where only detergents are excluded. Figure 9 shows the screening results, which once again demonstrates the high reproducibility achieved using His MultiTrap plates.

Summary

His MultiTrap FF and His MultiTrap HP 96-well plates are easy to use with either clarified or unclarified samples. They work smoothly for the purification screening of both membrane and soluble proteins. Reproducibility is excellent and purifications are directly scalable to larger columns. Short purification times help minimize adverse effects such as degradation and oxidation of sensitive target proteins.

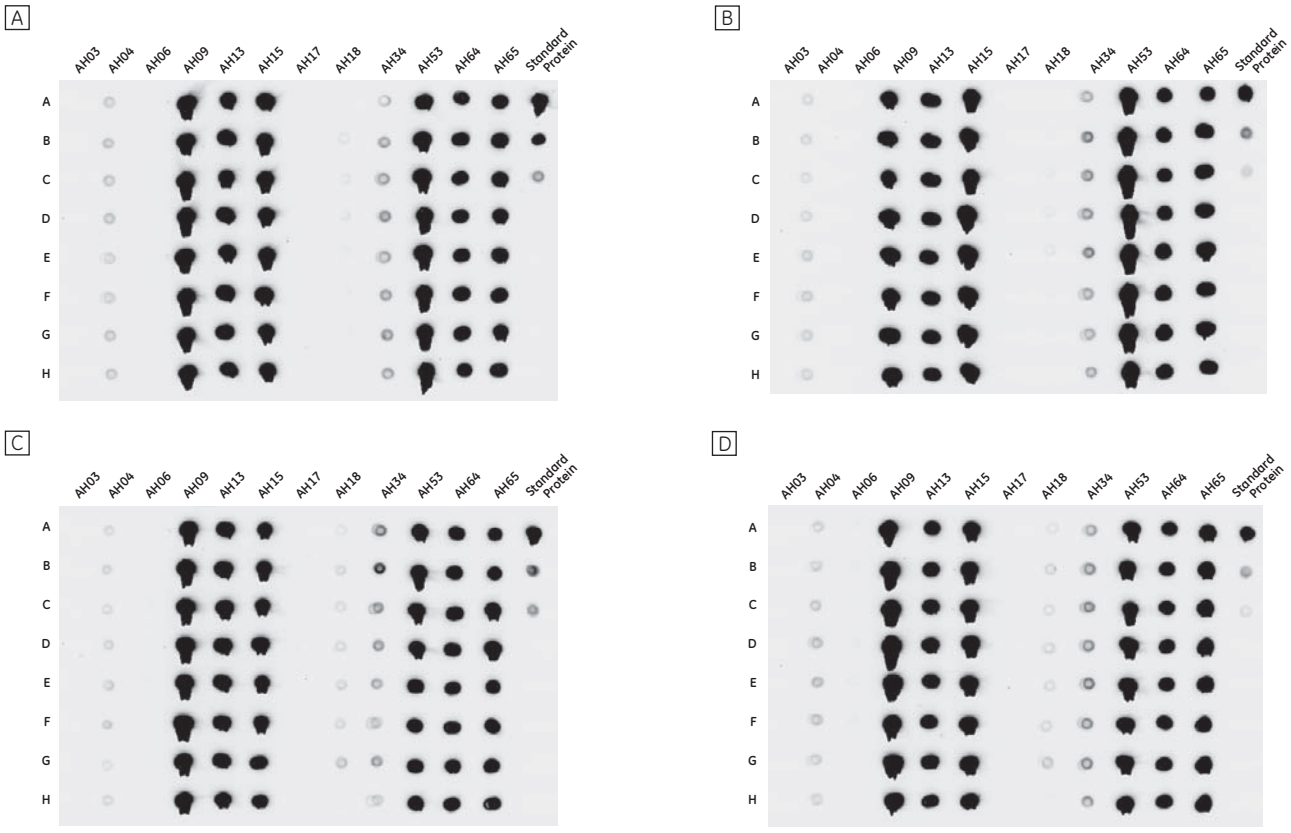

Fig 9. Dot-blots from screening of 12 soluble proteins with different expression levels with His MultiTrap FF (A and C) and His MultiTrap HP (B and D), Eight replicates were run for each protein. Only the first elution of each soluble protein is shown (50 µl elution volume).

Applications 2 to 4 were performed at the Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, by Victoria Lieu and Said Eshaghi.

Ordering information

Product	Quantity	Code no.
His MultiTrap FF	4 × 96-well plates	28-4009-90
His MultiTrap HP	4 × 96-well plates	28-4009-89
Collection plate (500 µl, V-bottom)	5 × 96-well plates	28-4039-43

Related products	Quantity	Code no.
His GraviTrap	10 × 1 ml	11-0033-99
His GraviTrap Kit*	1	28-4013-51
His Buffer Kit†	1	11-0034-00
His SpinTrap™	50 × 100 µl	28-4013-53
HisTrap FF	5 × 1 ml	17-5319-01
	5 × 5 ml	17-5255-01
HisTrap HP	5 × 1 ml	17-5247-01
	1 × 5 ml	17-5248-01
	5 × 5 ml	17-5248-02
HisTrap FF crude	5 × 1 ml	11-0004-58
	100 × 1 ml‡	11-0004-59
	5 × 5 ml	17-5286-01
	100 × 5 ml‡	17-5286-02
HisPrep FF 16/10	1 × 20 ml	17-5256-01
Ni Sepharose 6 Fast Flow	5 ml	17-5318-06
	25 ml	17-5318-01
	100 ml	17-5318-02
	500 ml§	17-5318-03
Ni Sepharose High Performance	25 ml	17-5268-01
	100 ml§	17-5268-02

* Contains two packs His GraviTrap and one pack His Buffer Kit.

† Contains phosphate buffer concentrates and highly pure 2 M imidazole stock solutions for His GraviTrap.

‡ Pack size available by special order.

§ Larger quantities are available. Please contact GE Healthcare for more information.

For contact information for your local office, please visit, www.gelifesciences.com/contact

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