cytiva

His **MultiTrap** FF and His **MultiTrap** HP

96-well plates

Instructions for Use

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1 Introduction

Intended use

This product is intended for research use only, and must not be used in any clinical or in vitro procedures for diagnostic purposes.

Principle

His MultiTrap™ FF and His MultiTrap HP are prepacked disposable 96-well filter plates for reproducible high throughput parallel purification of histidine-tagged recombinant proteins by Immobilized Metal ion Affinity Chromatography (IMAC). The 96-well filter plates are prepacked with precharged Ni Sepharose™ 6 Fast Flow and Ni Sepharose High Performance, respectively.

These plates simplify the purification screening and small scale purification of up to 1 mg of histidine-tagged proteins using centrifugation and up to 0.5 mg using vacuum.

After thorough cell disruption, it is possible to apply the unclarified lysate directly to the wells in the 96-well plate without precentrifugation and/or filtration of the sample. It is recommended to extend the lysis time if the sample is too viscous after lysis.

Prepacked 96-well filter plates give a reproducibility in yield and purity between wells and plates. His MultiTrap FF and His MultiTrap HP can be operated in robotic system or manually by centrifugation or vacuum. The purification protocol can easily be scaled up since Ni Sepharose is available in larger prepacked formats: HisTrap™ HP and HisTrap FF 1 and 5 mL columns and HisPrep™ FF 16/10 20 mL column (for order information see *Chapter 8 Ordering information, on page 29*).

Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance have low nickel ion (Ni^{2+}) leakage and are compatible with a wide range of additives used in protein screening purification. The design of the 96-well filter plate in combination with the resin, provides fast, simple and convenient parallel purifications. A short purification time generally minimizes deleterious effects, such as degradation and oxidation of sensitive target proteins.

2 Characteristics

His MultiTrap FF and His MultiTrap HP (See Figure below) are prepacked with the affinity resin Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance, respectively. The affinity resin consist of highly cross-linked agarose beads with an immobilized chelating group. The resin have been pre-charged with Ni^{2+} ions. The 96-well filter plates with 800 µL wells are made of polypropylene and polyethylene. In the Table below characteristics of His MultiTrap FF and His MultiTrap HP are presented.

Several amino acid residues, for example histidine residues, form complexes with metal ions. Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance selectively bind proteins if suitable complex-forming amino acid residues are exposed on the protein surface. An added polyhistidine tag increases the affinity for Ni^{2+} and makes the histidine-tagged protein the strongest binder among all the proteins in a crude sample extract, for example, a cell lysate.

Figure 2.1: His MultiTrap 96-well filter plates

Table 2.1: His MultiTrap FF and His MultiTrap HP characteristics

Filter plate size	127.8 × 85.5 × 30.6 mm according to ANSI/SBS 1-2004, 3-2004 & 4-2004 standards
Filter plate material	Polypropylene and polyethylene

Matrices	*His MultiTrap FF:* Ni Sepharose 6 Fast Flow; Highly cross-linked spherical agarose, 6%, precharged with Ni^{2+} ions *His MultiTrap FF:* Ni Sepharose High Performance; Highly cross-linked spherical agarose, precharged with Ni^{2+} ions
Average bead size	*Sepharose 6 Fast Flow:* 90 µm *Sepharose High Performance:* 34 µm
Metal ion capacity	~15 µmol Ni^{2+}/mL resin
Binding capacity[1]	*His MultiTrap FF:* Up to 0.8 mg histidine-tagged protein/ well *His MultiTrap HP:* Up to 1 mg histidine-tagged protein/ well Binding capacity might differ depending on proteins
Reproducibility between wells[2]	± 10%
Volume packed resin/well	50 µL (500 µL of 10% slurry)
Well volumes	800 µL
Number of wells	96
Centrifugation speed recommended: maximum:	Depends on sample pretreatment and sample properties. 100 to 500 × *g* 700 × *g*
Vacuum recommended: maximum:	Depends on sample pretreatment and sample properties. - 0.1 to - 0.3 bar - 0.5 bar
Compatibility with additives	Stable in all commonly used buffers, reducing agents, denaturants and detergents. See *Table 2.2, on page 6*.

Chemical stability[3]	0.01 M HCl or 0.1 M NaOH for one week at 40 ºC. 1 M NaOH or 70% acetic acid for 12 h. 2% SDS for 1 h. 30% 2-propanol for 30 min.
Avoid in buffers	Chelating agents, e.g., EDTA, EGTA, citrate. See Table below.
pH stability[3]	Short term (2 h): 2 to 14
	Long term (one week): 3 to 12
Storage solution	20% ethanol
Storage temperature	2ºC to 8ºC

[1] Optimum yield obtained with protein loads of up to 0.4 or 0.5 mg per well using His MultiTrap FF or His MultiTrap HP, respectively. Yield can be increased by a decrease of the imidazole concentration but this is at the expense of purity. When vacuum is used for aspiration of protein < 0.5 mg protein should be bound to resin. Risk for poor reproducibility and/or cross-contamination in form of foam in collection plate if higher amount of proteins are eluted.

[2] The amount of eluted target proteins/well does not differ more than ± 10% from the average amount/well for the whole filter plate.

[3] Ni^{2+}-stripped resin.

The Ni^{2+}-charged resin is compatible with all commonly used aqueous buffers, reducing agents, denaturants, such as 6 M guanidine-HCl (Gua-HCl) and 8 M urea, and a range of other additives (see Table below).

Table 2.2: Purification on Ni Sepharose 6 Fast Flow or Ni Sepharose High Performance can be performed in the presence of the following compounds at least at the concentrations given.

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
	6 M guanidine-HCl
Detergents	2% Tween™ 20 (nonionic)
	2% NP-40 (nonionic)
	2% cholate (anionic)
	1% CHAPS (zwitterionic)

Other additives	500 mM imidazole (will elute bound protein)
	20% ethanol
	50% glycerol
	100 mM Na_2SO_4
	1.5 M NaCl
	1 mM EDTA[2]
	60 mM citrate[2]
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[2]

[1] Do not leave MultiTrap plates with buffers including reducing agents when not in use.

[2] The strong chelator EDTA has been used successfully in some cases at 1 mM. Chelating agents must be used with caution and only in the sample (not in the buffers). Stripping of metal ions can be counteracted by addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects might vary with applied sample volume.

3 Advice on handling

Unclarified cell lysate

His MultiTrap FF and His MultiTrap HP are designed to allow parallel purification of histidine-tagged proteins directly from unclarified cell lysates. Sample preparation is performed by mechanical and/or chemical lysis. No centrifugation or filtration is needed before loading the sample onto the 96-well filter plate. If the sample is too viscous, an extension of the duration of mechanical treatment of the sample to make sure a more complete lysis is recommended (keep the sample on ice to prevent overheating).

Lysation with commercial lysis kits could give incomplete degradation of the cell paste which might result in problems when removing the sample from the wells. Therefore, if problems with draining the wells occur, centrifuge/filtrate the sample before adding it to the wells.

Metal ion

His MultiTrap FF and His MultiTrap HP are supplied precharged with Ni^{2+} ions. In general, Ni^{2+} is the preferred metal ion for purification of recombinant histidine-tagged proteins.

Buffers

We recommend binding at neutral to slightly alkaline pH (pH 7 to 8) in the presence of 0.5 to 1.0 M NaCl. Sodium phosphate buffers are often used. Tris-HCl can generally be used, but must be avoided in cases where the metal-protein affinity is weak, since it can reduce binding strength. Avoid chelating agents such as EDTA or citrate in buffers (see *Table 2.2, on page 6*). Imidazole is usually used for elution of histidine-tagged proteins.

Including salt in the buffers and samples eliminates ion-exchange effects but can also have a marginal effect on the retention of proteins.

Membrane proteins are usually purified in the presence of a detergent in the sample and buffers. Notice that the NaCl concentration might have to be optimized to avoid precipitation. Proteins expressed as inclusion bodies can be solubilized in denaturants such as urea or guanidine-HCl. The solubilized and denatured protein can then be purified in the presence of the denaturant.

Imidazole

Imidazole at low concentration is commonly used in the binding buffer as well as in the sample to minimize unspecific binding of host cell proteins. At somewhat higher concentrations, imidazole also decreases the binding of histidine-tagged proteins. It might therefore be necessary to optimize the imidazole concentration to ensure the best balance of high purity (low binding of host cell proteins), and high yield (strong binding of histidine-tagged target protein).

The optimal imidazole concentration in the binding buffer is protein-dependent, and is usually slightly higher for Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance than for similar IMAC resins on the market (see *Data File 11000886*). Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and-error effort, but 20 to 40 mM in the binding buffer as well as in the sample is a good starting point for many proteins.

Use high purity imidazole, which gives essentially no absorbance at 280 nm.

Alternative elution solutions

As alternatives to imidazole elution, histidine-tagged proteins can be eluted from the resin by other methods or combinations of methods, for example, lowering of pH to approximately pH 4.5. Below pH 4, metal ions will be stripped off the resin.

Note: *If the protein is sensitive to low pH, it is recommended to collect the eluted fractions in a collection plate containing 1 M Tris-HCl, pH 9.0 (60–200 µL/well) to restore the pH to neutral.*

EGTA and EDTA will strip metal ions from the resin and thereby cause protein elution. The co-eluted Ni^{2+} ions will remain chelated in the protein solution.

Recommended buffers

Recommended binding and elution buffers are listed in the Table below. The buffers can easily be prepared from His Buffer Kit or according to the description in *Chapter 6 Prepare buffers with different imidazole concentrations, on page 21*.

Table 3.1: Recommended buffers

Binding buffer	20 mM sodium phosphate, 500 mM NaCl, 20–40 mM imidazole, pH 7.4 (The optimal imidazole concentration is protein-dependent; 20–40 mM is suitable for many proteins).
Elution buffer	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4 (The imidazole concentration required for elution is protein-dependent).

If the recombinant histidine-tagged protein is expressed as inclusion bodies, include 6 M Guanidine-HCl (Gua-HCl) or 8 M urea in all buffers and sample to promote protein solubilization and unfolding. Refolding of the denatured protein might be possible, but depends on the protein. Advice for overcoming problems associated with inclusion bodies is found in *Chapter 7 Troubleshooting, on page 23*.

Tip: *Samples containing urea can be analyzed directly by SDS-PAGE whereas samples containing Gua-HCl must be buffer-exchanged to a buffer with urea before SDS-PAGE.*

Scaling up

Scaling up from His MultiTrap plates to a HisTrap 1 mL or 5 mL column while keeping the same conditions (e.g., Fast Flow or High performance resin, imidazole concentration etc.) provides highly consistent results and shortens the optimization time at scale up.

Ni Sepharose High Performance is recommended when high resolution and high capacity are important, whereas Ni Sepharose 6 Fast Flow is recommended when scale up is needed.

4 Sample preparation

For optimal growth and induction, refer to established protocols.

It is recommended that the samples are prepared according to standard protocols (see also below). After mechanical and/or chemical lysation the sample can be applied directly to the wells without clarification, excluding the centrifugation and/ or filtration steps (this applies to both His MultiTrap FF and His MultiTrap HP).

Recommended four-step protocol for cell lysis

The protocol below has been used successfully in our own laboratories for lysation of *E. coli*, but other established procedures might also work.

Step	Action
1	**Dilution of cell paste** • Add 5 to 10 mL of binding buffer for each gram of cell paste. • To prevent the binding of host cell proteins (with exposed histidines), it is essential to include imidazole at a low concentration in the sample and binding buffer (see *Chapter 6 Prepare buffers with different imidazole concentrations, on page 21*).
2	**Enzymatic lysis** • Add to final concentration: 0.2 mg/mL lysozyme, 20 µg/mL DNAse, 1 mM $MgCl_2$, 1 mM Pefabloc™ SC or PMSF. • Stir for 30 min at room temperature or + 4°C depending on the sensitivity of the target protein.

Step	Action
3	**Mechanical lysis** • Sonication on ice, approximately 10 min, *or* homogenization with a French press or other homogenizer, *or* freeze/thaw, repeated at least five times. ***Note:*** *Mechanical lysis time might have to be extended compared with standard protocols to secure an optimized lysate for sample loading (to prevent clogging of the wells). Different proteins have different sensitivity to cell lysis and caution has to be taken to avoid frothing and overheating of the sample.* ***Note:*** *Commercial lysis kits could also be used replacing the enzymatic and Mechanical lysis. However, poor disruption of cells can be obtained with more viscose sample as a result if the lysis is not well performed.*
4	**Adjustment of the pH of the lysate** • Measure and adjust pH if needed. Do not use strong bases or acids for pH-adjustment (due to precipitation risk). • Apply the unclarified lysate to the wells in the 96-well filter plate **directly** after preparation. ***Note:*** *Unclarified cell lysate might precipitate when not used immediately or frozen before use. New lysation of the sample can then prevent clogging of the wells when loading the 96-well filter plate.*

5 Protein purification protocols

There are two different protocols using either centrifugation (*Section 5.1 Centrifugation protein purification protocol, on page 14*) or vacuum pressure (*Section 5.3 Vacuum pressure protein purification protocol, on page 17*).

In this chapter

5.1 Centrifugation protein purification protocol

General considerations

- This protocol is only a general guideline for the purification with His MultiTrap FF and His MultiTrap HP. Optimization might be required depending on source and type of protein.
- Each well of the prepacked His MultiTrap FF and His MultiTrap HP has a capacity of up to 0.8 and 1.0 mg of polyhistidine-tagged protein, respectively. To obtain maximum yield, do not load more than 0.4 and 0.5 mg/well, respectively.
- In order to increase the purity, higher concentration of imidazole can be used in the sample and binding buffer.
- Mix briefly before centrifugation in the equilibration, wash and elution steps to increase the efficiency of the step.
- Do not apply more than 700 × *g* during centrifugation.
- Each well contains 500 µL 10% slurry of Ni Sepharose Fast Flow or Ni Sepharose High Performance in storage solution (50 µL resin in 20% ethanol).

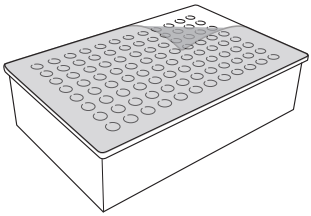
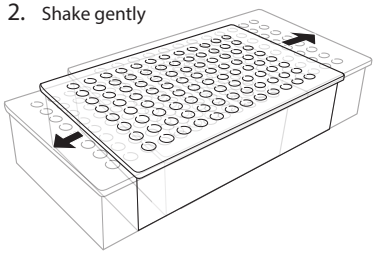
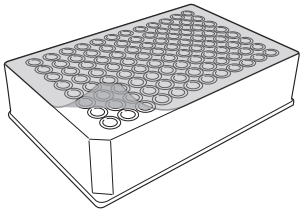
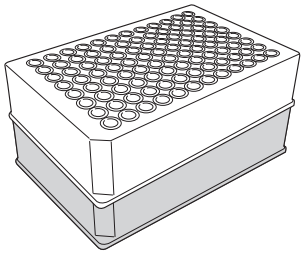
See *Section 5.2 Cue card: Centrifugation protocol work flow, on page 16* for a summary of the centrifugation protocol work flow.

Step	Action
1	Peel off the bottom seal. ***Note:*** *Hold the plate above a sink due to risk of small leakage of storage solution when removing the bottom seal.*
2	Gently shake the 96-well filter plate while holding it upside down, to remove any resin stuck on the top seal. Place the plate in upright position.
3	Peel off the top seal from the plate while holding it against the bench surface.
4	Position the plate on top of a collection plate. ***Note:*** *Remember to change or empty the collection plate when necessary during the following steps.*
5	Centrifuge the plates for 2 min at 500 × *g* to remove the storage solution from the resin.
6	Add 500 µL deionized water/well. Centrifuge for 2 min at 500 × *g*.

Step	Action
7	Add 500 µL binding buffer/well and mix briefly to equilibrate the resin. Centrifuge for 2 min at 500 × *g*. Repeat once. ***Note:*** *Reducing agents can be used in sample and buffers. Do not leave His MultiTrap plates with buffers including reducing agents when not in use.*
8	Apply unclarified or clarified lysate (maximum 600 µL/well) to the wells, mix briefly, and incubate for 3 min. (Increase the incubation time if the yield is too low).
9	Remove the flow through by centrifuging for 4 min at 100 × *g* (or until all wells are empty).
10	Add 500 µL binding buffer/well and mix briefly to wash out unbound sample. Centrifuge at 500 × *g* for 2 min. Repeat once (or until all unbound sample are removed, A_{280} should be < 0.1 for high purity).
11	Add 200 µL[1] of elution buffer/well and mix for 1 min. Change collection plate and centrifuge the plates at 500 × *g* for 2 min and collect the fractions. Repeat twice (or until all target protein has been removed, A_{280} < 0.1 for high purity). If required, change collection plate between each elution (to prevent unnecessary dilution of the target protein).

[1] The volumes can be varied depending on which concentration of target protein needed, for example, 50 or 100 µL elution buffer/well.)

5.2 Cue card: Centrifugation protocol work flow

1. Remove bottom seal 	2. Shake gently 
3. Remove top seal 	4. Place on collection plate 
5. 500 x g for 2 min	6. + 500 µL water/well 500 x g for 2 min
7. + 500 µL binding buffer/well, mix briefly 500 x g for 2 min } repeat twice	8. + up to 600 µL unclarified lysate/well , mix breifly Incubate for 3 min
9. 100 x g for 4 min	10. + 500 µL binding buffer/well, mix briefly 500 x g for 2 min } repeat twice
11. New collection plate + x µL elution buffer/well, mix 1 min 500 x g for 2 min } repeat twice	

5.3 Vacuum pressure protein purification protocol

General considerations

- This protocol is only a general guideline for the purification with His MultiTrap FF and His MultiTrap HP. Optimization might be required depending on source and type of protein.
- Each well of the prepacked His MultiTrap FF and His MultiTrap HP has a capacity of up to 0.8 and 1.0 mg of polyhistidine-tagged protein, respectively.

  To obtain maximum results, do not load more than 0.4 and 0.5 mg/well, respectively. Loads > 0.5 mg can give poor reproducibility and/or cross-contamination in form of foam in collection plate.
- In order to increase the purity, higher concentration of imidazole can be used in the sample and binding buffer.
- Mix briefly before vacuum pressure in the equilibration, wash and elution steps to increase the efficiency of the step.
- Do not apply more vacuum than - 500 mbar.
- If problems with foaming, reproducibility or bubbles in the collection plate occur using vacuum, decrease load of protein (< 0.5 mg protein bound to resin). If this is not working, the centrifugation protocol should be considered.
- To avoid cross contamination, the distance between MultiTrap and collection plate must not be more than 5 mm.

  Use deep well collection plates (500 µL) to avoid splashes between wells. A vacuum pressure of -150 mbar (40 s) followed by - 300mbar (3 s) must be used during elution of purified protein.
- Each well contains 500 µL 10% slurry of Ni Sepharose Fast Flow or High Performance in storage solution (50 µL resin in 20% ethanol).

See *Section 5.4 Cue Card: Vacuum pressure protocol work flow, on page 20* for a summary of the vacuum pressure protocol work flow.

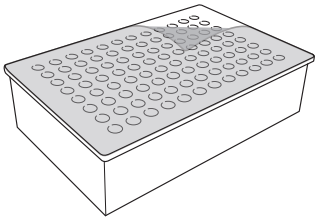
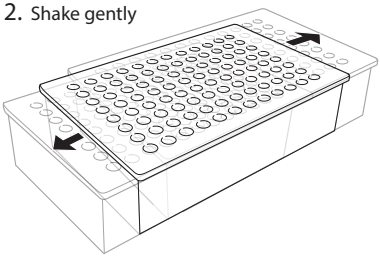
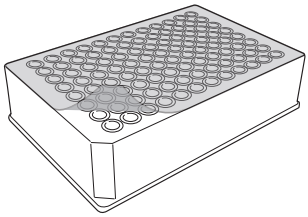
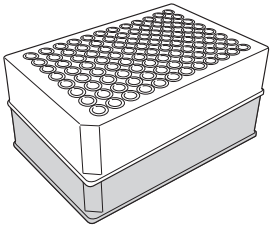
Step	Action
1	Peel off the bottom seal. ***Note:*** *Hold the plate above a sink due to risk of small leakage of storage solution when removing the bottom seal.*
2	Gently shake the 96-well filter plate while holding it upside down, to remove any resin stuck on the top seal. Place the plate in up-right position.

Step	Action
3	Peel off the top seal from the plate while holding it against the bench surface.
4	Place the 96-well filter plate on the vacuum manifold. Remove the ethanol from the resin by applying a vacuum pressure of - 300 mbar for 30 s. ***Note:*** *Position the filter plate on top of a collection plate if the solution not is removed from manifold by the vacuum. Remember to change or empty the collection plate when necessary during the following steps.*
5	Add 500 µL deionized water/well. Remove the water from the wells by applying a vacuum pressure of - 300 mbar for 30 s.
6	Add 500 µL binding buffer/well and mix briefly to equilibrate the resin. Remove the solution by applying a vacuum pressure of - 300 mbar for 30 s. Repeat once. ***Note:*** *Reducing agents might be used in sample and buffers. Do not leave His MultiTrap plates with buffers including reducing agents when not in use.*
7	Apply unclarified or clarified lysate (maximum 600 µL/well) to the wells, mix briefly and incubate for 3 min. (Increase the incubation time if the yield is too low.) ***Note:*** *In purifications using robot, the vacuum has to be adjusted to methods applicable to the robot.*
8	Remove the flow through by applying a vacuum pressure of - 150 mbar until all wells are empty (it takes approximately 1 min).
9	Add 500 µL binding buffer/well and mix briefly to wash out unbound sample. Remove the solution applying a vacuum pressure of - 300 mbar for 45 s. Repeat 2 times (or until all unbound sample are removed, A_{280} should be < 0.1 for high purity).

Step	Action
10	Add 200 µL elution buffer[2] and mix for 1 min. Change/add collection plate and elute the sample using vacuum by applying a vacuum pressure of - 160 mbar for 45 s followed by applying a vacuum pressure of - 300 mbar for 2 s. Repeat once (or until all target protein and additives has been removed). If required, change collection plate between each elution to prevent unnecessary dilution of the target protein. ***Note:*** *Increasing the vacuum too fast can give foam under the filter plate and cross contamination can occur.*

[2] The volumes can be varied depending on which concentration of target protein needed, for example, 50 or 100 µL elution buffer/well.

5.4 Cue Card: Vacuum pressure protocol work flow

1. Remove bottom seal 	2. Shake gently 
3. Remove top seal 	4. Vacuum: -300 mbar If required place on collection plate 
5. Add 500 µL water/well Vacuum: - 300 mbar	6. Add 500 µL binding buffer/well, mix briefly Vacuum: - 300 mbar (20-30 s) } repeat once
7. Add up to 600 µL unclarified lysate/well, mix briefly Incubate for 3 min	8. Vacuum: first -160 mbar (40 s), second - 300 mbar (3 s)
9. Add 500 µL binding buffer/well, mix briefly Vacuum: - 300 mbar } repeat twice	10. Place on new collection plate Add x µL elution buffer/well, mix 1 min Vacuum: -160 mbar (45 s) Vacuum: -300 mbar (2 s) } repeat twice

6 Prepare buffers with different imidazole concentrations

Phosphate buffer (containing imidazole for binding and elution buffers)

Prepare 1 liter of 20 mM sodium phosphate, 500 mM NaCl, 10 to 500 mM imidazole, pH 7.4:

Step	Action
1	Add the following into a calibrated bottle: • 1.78 g $Na_2HPO_4 \times 2H_2O$ (177.99 g/mol) • 1.38 g $NaH_2PO_4 \times H_2O$ (137.99 g/mol) • 29.22 g NaCl (58.44 g/mol) • X g imidazole (68.08 g/mol) (depending on the chosen imidazole binding and elution concentrations, see Table below)
2	Add distilled water to 900 mL and dissolve completely.
3	Adjust pH from basic to 7.4 with HCl.
4	Add distilled water to 1000 mL and filter through a 0.45 µm filter.

Table 6.1: Concentration and weight of imidazole in phosphate buffer

Imidazole[1] concentration in buffer [mM]	**X = Weight of imidazole in phosphate buffer [g]**
10	0.7
20	1.4
30	2.0
40	2.7
50	3.4
60	4.1
70	4.8
80	5.4

90	6.1
100	6.8
200	13.6
300	20.4
400	27.2
500	34.0

[1] Use high purity imidazole as this will give very low or no absorbance at 280 nm.

7 Troubleshooting

The following tips might be of assistance.

If you have any further questions about your His MultiTrap FF or His MultiTrap HP 96-well filter plates, contact your local Cytiva representative.

Fault	Possible cause	Action
The wells are clogging	The sample is too viscous	• Increase dilution of the cell paste before lysis, or dilute after the lysation. • Increase time for lysis until the viscosity is reduced, and/or add an additional dose of DNAse and Mg^{2+}. • Centrifuge and/or filtrate the sample if unclarified sample has been used. • If the purification has been performed at + 4°C, move to room temperature if possible.

	Protein is difficult to dissolve or precipitates during purification	• Add detergents, reducing agents or other additives to the sample (see *Table 2.2, on page 6*) and mix gently for 30 min to solubilize the tagged protein. Note that NP-40, but not Tween, has a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange. • Inclusion bodies: the protein can usually be solubilized (and unfolded, refolding needed to obtain active protein) from inclusion bodies using common denaturants such as 4 to 6 M Gua-HCl, 4 to 8 M urea, or strong detergents. Mix gently for 30 minutes or more to aid solubilization of the tagged protein. Purify in the presence of the denaturant. • If possible, decrease the NaCl concentration in the elution buffer. Adjust ion strength or pH of sample.

Leakage of solution after removing foils	-	• Add 500 µL deionized water before adding binding buffer to the wells. Remove the solution between the additions with either centrifugation or vacuum.
Problem with reproducibility and/or cross contamination cause by foam and/or splash in collection plate when using vacuum	Distance between filter plate and collection plate to large. Too high vacuum pressure.	• Distance between filter plate and collection plate must not be more than 5 mm. • Increase first elution time if solution is not aspirated from well. If that is not working increase vacuum. • Use deep well collection plate to avoid splashes in to adjacent wells. • Decrease vacuum if splashes of solution into adjacent wells occurred. • Add more wash steps before eluting the protein. • Change to centrifugation.

Low yield of histidine-tagged protein	Protein found in the flow through during sample application and wash	• Imidazole concentration in sample and binding buffer is too high. Use lower concentration. • Increase the incubation time of the sample in the wells and use lower centrifugation speed/ vacuum when removing the sample. • Make sure that the concentration of chelating or strong reducing agents in the sample is not too high. • Histidine tag might be insufficiently exposed; perform purification of unfolded protein in urea or Gua-HCl as for inclusion bodies. To minimize dilution of the sample, solid urea or Gua-HCl can be added. • Histidine tag has been lost. Check sequence of the construct or Western blot of extract using anti-His antibody.

	Protein is not eluted during purification	• Histidine-tagged protein still bound. Elute with higher concentration of imidazole in the elution buffer. • Protein has precipitated in the wells. Decrease amount of protein loaded to the wells. Decrease imidazole concentration during elution. Try changing NaCl concentration and pH. Add detergents or elute under denaturing (unfolding) conditions. • Non-specific hydrophobic or other interaction. Add a nonionic detergent to the elution buffer or increase/decrease the NaCl concentration.
Eluted protein is not pure	Too low imidazole concentration in sample and binding buffer	• Use higher imidazole concentration in sample and binding buffer to avoid binding of contaminants. 20 to 40 mM is recommended, but higher concentrations might also be appropriate (protein dependent).

Partial degradation of tagged protein by proteases	• Add protease inhibitors (but use EDTA with caution, see *Table 2.2, on page 6*). • Work at low temperature.
Contaminants are associated with tagged proteins	• Add detergent and/or reducing agents before sonicating the cells. Increase detergent levels (e.g., 2% Tween), or add glycerol (up to 50%) to the wash buffer to disrupt non-specific interactions.
Unbound material is not sufficiently washed off	• Repeat the wash step after sample application to obtain optimal purity.

8 Ordering information

Items	Quantity	Product code
His MultiTrap FF	4 × prepacked 96-well plates	28400990
His MultiTrap HP	4 × prepacked 96-well plates	28400989

Related products	Quantity	Product code
His Buffer Kit	1	11003400
His GraviTrap™	10 × 1 mL	11003399
His GraviTrap Kit	1	28401351
His SpinTrap™	50 x 100 µL	28401353
His SpinTrap Kit	1	28932171
HisTrap HP	5 x 1mL	17524701
HisTrap HP	1 x 5 mL	17524801
HisTrap HP	5 x 5 mL	17524802
HisTrap FF	5 x1 mL	17531901
HisTrap FF	5 x 5 mL	17525501
HisPrep FF 16/10	1 x 20 mL	17525601

Accessories	Quantity	Product code
Collection plate 96-well plate 500 µL, V-shaped bottom	5 pack	28403943

Literature	Product code
Ni Sepharose and IMAC Sepharose, Selection Guide	28407092
Affinity Chromatography Columns and Resin Product Profile	18112186
Affinity Chromatography Handbook, Principle and Methods	18102229

Recombinant Protein Handbook, Protein Amplification and Simple Purification	18114275
Data file His MultiTrap FF and His MultiTrap HP	11003663
The Trap platform and Tecan automation - efficient solutions for screening proteins	28928959

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