GE Healthcare

Instructions 11-0036-62 AB

His MultiTrap FF and His MultiTrap HP

MultiTrap

Contents

1 Intended use

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

2 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 pre-charged 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 pre-centrifugation 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 page 22).

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 media, provides fast, simple and convenient parallel purifications. A short purification time generally minimizes deleterious effects, such as degradation and oxidation of sensitive target proteins.

3 Characteristics

His MultiTrap FF and His MultiTrap HP (Fig. 1) are prepacked with the affinity media Ni Sepharose 6 Fast Flow and Ni Sepharose High Performance, respectively. The affinity media consist of highly cross-linked agarose beads with an immobilized chelating group. The media have been pre-charged with Ni^{2+} ions. The 96-well filter plates with 800 µl wells are made of polypropylene and polyethylene. In Table 1 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, *e.g.* a cell lysate.

Fig. 1. His MultiTrap 96-well filter plates

Table 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%, pre-charged with Ni^{2+} ions *His MultiTrap FF:* Ni Sepharose High Performance; Highly cross-linked spherical agarose, pre-charged 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 medium
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 may differ depending on proteins
Reproducibility between wells[2]	±10%
Volume packed medium/well	50 µl (500 µl of 10% slurry)
Well volumes	800 µl
Number of wells	96
Centrifugation speed recommended: maximum:	Depends on sample pre-treatment and sample properties. 100 to 500 × *g* 700 × *g*
Vacuum recommended: maximum:	Depends on sample pre-treatment 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.
Chemical stability[3]	0.01 M HCl or 0.1 M NaOH for one week at 40 °C. 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 2.
pH stability[3]	Short term (2 h): 2–14 Long term (one week): 3–12
Storage solution	20% ethanol
Storage temperature	+4 to +30 °C

[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 medium. 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 media.

The Ni^{2+}-charged medium 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 2).

Table 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% Triton™ X-100 (nonionic) 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[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[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 should be used with caution and only in the sample (not in the buffers). Stripping of metal ions may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with applied sample volume.

4 Advice on handling

4.1 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 ensure 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 may 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.

4.2 Metal ion

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

4.3 Buffers

We recommend binding at neutral to slightly alkaline pH (pH 7–8) in the presence of 0.5–1.0 M NaCl. Sodium phosphate buffers are often used. Tris-HCl can generally be used, but should be avoided in cases where the metal-protein affinity is weak, since it may reduce binding strength. Avoid chelating agents such as EDTA or citrate in buffers (see Table 2). 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 may 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.

4.4 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 may 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 media on the market (see Data File 11-0008-86). Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and-error effort, but 20-40 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.

4.5 Alternative elution solutions

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

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 medium and thereby cause protein elution. The co-eluted Ni^{2+} ions will remain chelated in the protein solution.

4.6 Recommended buffers

Recommended binding and elution buffers are listed in Table 3. The buffers can easily be prepared from His Buffer Kit or according to the description in Section 7.

Table 3. 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 may be possible, but depends on the protein. Advice for overcoming problems associated with inclusion bodies is found in section 8, Troubleshooting.

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.

4.7 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 medium, 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.

5 Sample preparation

For optimal growth and induction, please 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 may also work.

1 Dilution of cell paste

- Add 5–10 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 section 7, Prepare buffers with different imidazole concentrations).

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 minutes at room temperature or +4 °C depending on the sensitivity of the target protein.

3 Mechanical lysis

- Sonication on ice, approximately 10 minutes,
  or
  homogenization with a French press or other homogenizer,
  or
  freeze/thaw, repeated at least five times.

Note: *Mechanical lysis time may 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 may 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 may 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.*

6 Protein purification protocols

There are two different protocols using either centrifugation (Section 6.1) or vacuum pressure (Section 6.2).

6.1 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 may 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.
- 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 medium in 20% ethanol).

See Fig. 2 for a summary of the centrifugation protocol work flow.

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 medium stuck on the top seal. Place the plate in up-right 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 minutes at 500 × *g*, to remove the storage solution from the medium.

6 Add 500 µl deionized water/well.
Centrifuge for 2 minutes at 500 × *g*.

7 Add 500 µl binding buffer/well, to equilibrate the medium.
Centrifuge for 2 minutes at 500 × *g*.
Repeat once.

Note: *Reducing agents may 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, incubate for 3 minutes. (Increase the incubation time and gently mix the filter plate if the yield is too low).

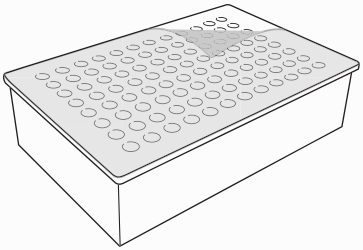
9 Remove the flow through by centrifuging for 4 minutes at 100 × *g* (or until all wells are empty).

10 Add 500 µl binding buffer/well to wash out unbound sample.
Centrifuge at 500 × *g* for 2 minutes.
Repeat once (or until all unbound sample are removed, A_{280} should be < 0.1 for high purity).

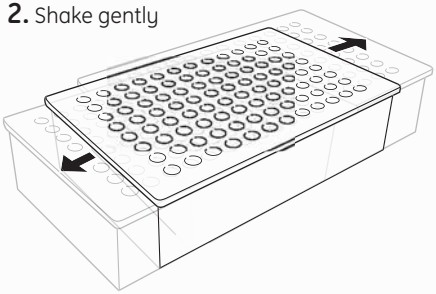
11 Add 200 µl[1] of elution buffer/well and mix for 1 minutes.
Change collection plate and centrifuge the plates at 500 × *g* for 2 minutes and collect the fractions.
Repeat twice (or until all target protein has been removed, A_{280} < 0.1 for high purity).
If required, change collection plate between each elution (to prevent unnecessary dilution of the target protein).

[1] The volumes can be varied depending on which concentration of target protein needed, *e.g.* 50 or 100 µl elution buffer/well.)

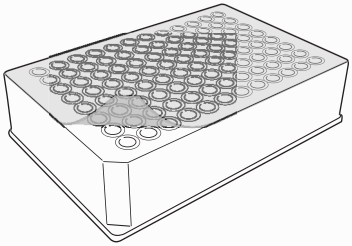
1. Remove bottom seal



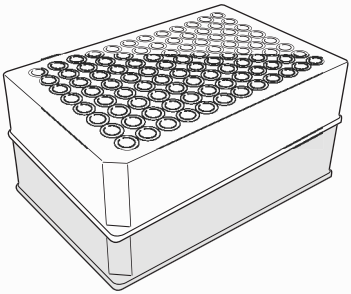
2. Shake gently



3. Remove top seal



4. Place on collection plate



5. 500 x *g* for 2 minutes

6. + 500 µl water/well
500 x *g* for 2 minutes

7. + 500 µl binding buffer/well
500 x *g* for 2 minutes
} repeat twice

8. + up to 600 µl unclarified lysate/well
Incubate for 3 minutes

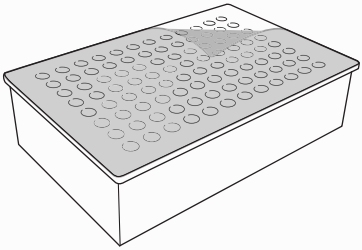
9. **100 x *g*** for 4 minutes

10. + 500 µl binding buffer/well
500 x *g* for 2 minutes
} repeat twice

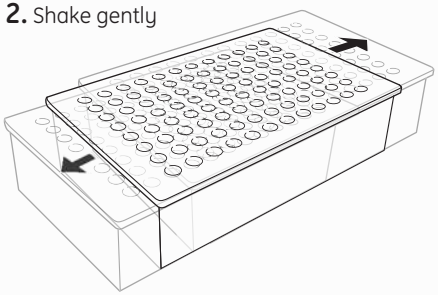
11. New collection plate
+ x µl elution buffer/well
Mix 1 minute
500 x *g* for 2 minutes
} repeat twice

Fig. 2. Centrifugation protocol work flow

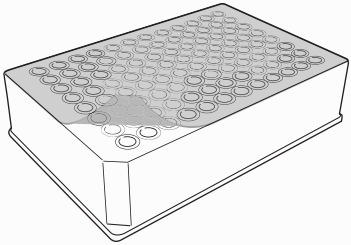
1. Remove bottom seal



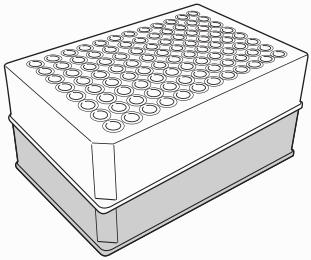
2. Shake gently



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
Vacuum: -300 mbar
} repeat twice

7. Add up to 600 µl unclarified lysate/well
Incubate for 3 minutes

8. Vacuum: -150 mbar

9. Add 500 µl binding buffer/well
Vacuum: -300 mbar
} repeat 3 X

10. Place on new collection plate
Add x µl elution buffer/well
Mix 1 minute
Vacuum: -150 mbar (40 s)
Vacuum: -30 mbar (3 s)
} repeat twice

Fig. 3. Vacuum pressure protocol work flow.

6.2 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 may 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 may 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.
- 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 (<0.5mg protein bound to medium). If this is not working, the centrifugation protocol should be considered.
- To avoid cross contamination, the distance between MultiTrap and collection plate should 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 sec) followed by -300mbar (3 sec) should 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 medium in 20% ethanol).

See Fig. 3 for a summary of the vacuum pressure protocol work flow.

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 medium stuck on the top seal. Place the plate in up-right position.

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 medium by applying a vacuum pressure of -300 mbar for 30 sec.

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 sec.

6 Add 500 µl binding buffer/well to equilibrate the medium.
Remove the solution by applying a vacuum pressure of -300 mbar for 30 sec.
Repeat once.

Note: *Reducing agents may 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, incubate for 3 minutes. (Increase the incubation time and gently mix the filter plate 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 minute).

9 Add 500 µl binding buffer/well to wash out unbound sample.
Remove the solution applying a vacuum pressure of -150 mbar for 40 sec.
Repeat 2 times (or until all unbound sample are removed, A_{280} should be < 0.1 for high purity).

10 Add 200 µl elution buffer[1] and mix for 1 minute.
Change/add collection plate and elute the sample using vacuum by applying a vacuum pressure of -150 mbar for 40 sec followed by applying a vacuum pressure of -300 mbar for 3 sec.
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.*

[1] The volumes can be varied depending on which concentration of target protein needed, *e.g.* 50 or 100 µl elution buffer/well.

7 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, 10–500 mM imidazole, pH 7.4:

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 4)

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 4. 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.

8 Troubleshooting

The following tips may be of assistance.
If you have any further questions about your His MultiTrap FF or His MultiTrap HP 96-well filter plates, please visit www.amershambiosciences.com/his, contact our technical support, or your local GE Healthcare 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) and mix gently for 30 minutes to solubilize the tagged protein. Note that Triton X-100 and NP-40 (but not Tween) have 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 4-6 M Gua-HCl, 4-8 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

Fault	Possible cause	Action
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 should 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 may 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 non-ionic detergent to the elution buffer or increase/decrease the NaCl concentration.

Fault	Possible cause	Action
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. 20-40 mM is recommended, but higher concentrations may also be appropriate (protein dependent).
	Partial degradation of tagged protein by proteases	• Add protease inhibitors (but use EDTA with caution, see Table 2). • 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.* up to 2% Triton X-100 or 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.

9 Ordering information

Items	No. supplied	Code no.
His MultiTrap FF	4 × prepacked 96-well plates	28-4009-90
His MultiTrap HP	4 × prepacked 96-well plates	28-4009-89

Related products	No. supplied	Code no.
His Buffer Kit	1	11-0034-00
His GraviTrap™	10 × 1 ml	11-0033-99
His GraviTrap Kit	1	28-4013-51
His SpinTrap™	50 x 100 µl	28-4013-53
His SpinTrap Kit	1	28-9321-71
HisTrap HP	5 x 1ml	17-5247-01
HisTrap HP	1 x 5 ml	17-5248-01
HisTrap HP	5 x 5 ml	17-5248-02
HisTrap FF	5 x1 ml	17-5319-01
HisTrap FF	5 x 5 ml	17-5255-01
HisPrep FF 16/10	1 x 20 ml	17-5256-01

Accessories	No. supplied	Code no.
Collection plate 96-well plate 500 µl, V-shaped bottom	5 pack	28-4039-43

Literature	Code no.
Ni Sepharose and IMAC Sepharose, Selection Guide	28-4070-92
Affinity Chromatography Columns and Media Product Profile	18-1121-86
Affinity Chromatography Handbook, Principle and Methods	18-1022-29
Recombinant Protein Handbook, Protein Amplification and Simple Purification	18-1142-75
Data file His MultiTrap FF and His MultiTrap HP	11-0036-63
The Trap platform and Tecan automation - efficient solutions for screening proteins	28-9289-59

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Licensing information
Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US pat 5,284,933 and US pat 5,310,663, including corresponding foreign patents (assigne: Hoffman La Roche, Inc).

This product is covered by US pat 6,623,655 and their equivalents in other countries.

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