GE Healthcare
Life Sciences

Instructions 29-0042-12 AB **Affinity chromatography**

His GraviTrap™ TALON®
His SpinTrap™ TALON
His MultiTrap™ TALON

His GraviTrap TALON, His SpinTrap TALON and His MultiTrap TALON are designed for rapid and simple small-scale purification and screening of histidine-tagged proteins.

- His GraviTrap TALON is a prepacked gravity flow column,
- His SpinTrap TALON is a prepacked spin column, and
- His MultiTrap TALON is a prepacked 96-well filter plate.

  All containing TALON Superflow™ chromatography medium.

All three formats are intended for purification of histidine-tagged proteins by immobilized metal affinity chromatography (IMAC). TALON Superflow is an IMAC medium precharged with cobalt, which allows purification of polyhistidine-tagged proteins during mild conditions, low imidazole concentrations in sample, wash and elution buffers, in order to preserve native structure while still giving high selectivity.

Intended use

His GraviTrap TALON, His SpinTrap TALON and His MultiTrap TALON are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

CAUTION!

Contains cobalt. May cause an allergic reaction.

Table of contents

1 Product description 3
2 Buffer recommendations 8
3 Protocols 10
4 Optimization 21
5 Tips and hints 21
6 Ordering Information 27

1 Product description

His GraviTrap TALON, His SpinTrap TALON and His MultiTrap TALON, contain TALON Superflow medium precharged with cobalt ions. The products are intended for rapid and simple manual small-scale purification and screening of histidine-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC).

The medium exhibits a reduced affinity for host proteins, has a low metal ion leakage and is compatible with commonly used IMAC reagents. It is appropriate for purifying proteins under native or denaturing conditions, allowing fast, simple and convenient purification of proteins.

Clarification of sample is recommended for His SpinTrap TALON and His MultiTrap TALON for higher purity and recovery. However, both clarified and unclarified sample can be applied on His GraviTrap TALON.

His GraviTrap TALON

His GraviTrap TALON is a prepacked, gravity-flow column containing 1 ml TALON Superflow chromatography medium and is designed for manual purification without the need for a purification system. One purification run takes approximately 30 minutes and both clarified and unclarified sample can be applied to the column.

Large sample volumes can be applied all at once, and the histidine-tagged protein is effectively eluted in a small volume.

The design of the GraviTrap column includes special frits protecting the medium from running dry during purification. Together with LabMate™ PD-10 Buffer Reservoir larger sample volumes up to 35 ml can be applied in one step with the gravity flow protocol (order separately). See Table 1 for the characteristic of His GraviTrap TALON.

His SpinTrap TALON

His SpinTrap TALON is a prepacked column containing 100 µl Talon Superflow chromatography medium suitable for purification of multiple samples in parallel, for example in screening experiments. The column can be used with a standard microcentrifuge and purification takes approximately 30 minutes.

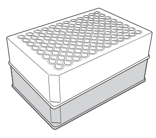
See Table 1 for the characteristics of His SpinTrap TALON.

Table 1. His GraviTrap TALON and His SpinTrap TALON characteristics.

Column material	Polypropylene barrel, polyethylene frits
Medium	TALON Superflow Cobalt charged affinity resin
Particle size distribution	60 to160 µm
Protein binding capacity[1]	
- His GraviTrap TALON	Approximately 15 mg histidine-tagged protein/column
- His SpinTrap TALON	Approximately 1 mg histidine-tagged protein/column
Bed volume	
- His GraviTrap TALON	1 ml
- His SpinTrap TALON	100 µl
Compatibility during use	Stable in all commonly used buffers, denaturants and detergents. (See Table 3)
Avoid in buffers	Chelating agents, e.g., EDTA, EGTA, citrate and DTT, DTE, TCEP.
pH stability[2]	
- short term (2 hours)	2 to 14
- long term (one week)	3 to 12
Storage	20% ethanol at 4°C to 8°C

[1] The binding capacity for individual proteins may vary.
[2] Co^{2+}-stripped media.

His MultiTrap TALON



His MultiTrap TALON are prepacked disposable 96-well filter plates for reproducible high throughput parallel screening of histidine-tagged proteins. Each well is prefilled with 50 µl TALON Superflow chromatography medium. His MultiTrap TALON can be operated both in robotic systems and manually by centrifugation or vacuum, giving a high reproducibility in yield and purity between wells and plates. The 96-well filter plate simplifies screening and small scale purification of up to 1 mg of histidine-tagged proteins.

The characteristics of His MultiTrap TALON are presented in Table 2.

Table 2. His MultiTrap TALON characteristics.

Filter plate material	Polypropylene, polyethylene
Filter plate size	127.8 × 85.5 × 30.6 mm according to ANSI/SBS 1-2004, 3-2004 and 4-2004 standards
Medium	TALON Superflow Cobalt charged affinity resin
Particle size distribution	60 to 160 µm
Protein binding capacity[1]	Up to 1 mg histidine-tagged protein/ well
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 pretreatment 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 during use	Stable in all commonly used buffers, denaturants and detergents. (See Table 3)
Avoid in buffers	Chelating agents, e.g., EDTA, EGTA, citrate and DTT, DTE, TCEP.
pH stability[3] - short term (2 hours) - long term (one week)	 2 to 14 3 to 12
Storage	20% ethanol at 4ºC to 8ºC

1 Optimum yield obtained with protein loads of up to 0.4 or 0.5 mg per well using His MultiTrap TALON. 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 due to foaming if higher amount of proteins are eluted. Binding capacity may differ depending on proteins.

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 Co^{2+} stripped media.

Compatibility

TALON Superflow chromatography medium is compatible with all commonly used aqueous buffers, denaturants such as 6 M Gua-HCl and 8 M urea, and a range of other additives (see Table 3).

Table 3. Compatible reagents for TALON Superflow[1].

Reagent	Acceptable Concentration
β-Mercaptoethanol[2]	10 mM (with caution)
CHAPS, SDS, sarcosyl[3]	1% (with caution)
Ethanol[4]	30%
Ethylene glycol	30%
HEPES	50 mM
Glycerol	20%
Guanidinium hydrochloride	6 M
Imidazole[5]	≤ 500 mM at pH 7.0 to 8.0, for elution
KCl	500 mM
MES	20 mM
MOPS	50 mM
NaCl	1.0 M
NP-40	1%
TRIS[6]	50 mM
Triton™ X-100	< 1%
Urea	8 M

1 Data provided by Clontech Laboratories, Inc.
2 Use TALON Superflow immediately after equilibrating with buffers containing β-Mercaptoethanol. Otherwise, the medium will change color. Do not store the medium in buffers containing β-Mercaptoethanol.
3 Ionic detergents like CHAPS (3-[(30Cholamidopropyl)-dimethylammonio]-1-propane-sulfonate), SDS (sodium dodecyl sulfate), and sarkosyl are compatible up to 1%. However, due to their charged nature, you should anticipate interference with binding.
4 Ethanol may precipitate proteins, causing low yields and column clogging.
5 Imidazole at concentrations higher than 5-10 mM may cause lower yields of histidine-tagged proteins, because it competes with the histidine side chains (imidazole groups) for binding to the immobilized metal ions.
6 TRIS coordinates weakly with metal ions, causing a decrease in capacity.

Avoid using the following reagents

- DTT (dithiothreitol), DTE (dithioerythritol) and TCEP (TRIS (2-carboxyethyl)phosphine). Protein binding capacity will decrease rapidly.

  Note: *Use of strong reducing agents will cause discoloring of the medium and will interfere with the binding of the cobalt metal ions to the chromatography medium.*

- EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene glycolbis([β-amino-ethyl ether]). These chelators will strip off the cobalt ions from the medium.

  Note: *Although EDTA can be used at indicated points, it must be removed from the sample by buffer exchange prior to application to TALON Superflow.*

2 Buffer recommendations

General

- The recommended binding buffer condition is at neutral to slightly alkaline pH (pH 7 to 8) in the presence of 0.3 M to 0.5 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 very weak, since it may reduce binding strength. Avoid chelating agents such as EDTA or citrate in buffers. In general, imidazole is used for elution of histidine-tagged proteins.
- Including salt in the buffers and samples, for example 0.3 M to 0.5 M NaCl, eliminates ion-exchange effects but can also have a marginal effect on the retention of proteins.
- Below pH 4, metal ions will be stripped off the medium.

- EGTA and EDTA will strip metal ions from the medium and thereby cause protein elution, but the target pool will then contain chelated Co^{2+} ions. In this case, the Co^{2+} ions can be removed by desalting on following columns and 96-well plate: Disposable PD-10 Desalting, PD-MidiTrap™ G-25, PD MiniTrap G-25, PD SpinTrap™ G-25 or PD MultiTrap™ G-25, see *Ordering Information*.

Native protein purification

- Binding buffer: 50 mM sodium phosphate, 300 mM NaCl[1], pH 7.4
- Wash buffer: 50 mM sodium phosphate, 300 mM NaCl[1], 5 mM imidazole[2], pH 7.4
- Elution buffer:50 mM sodium phosphate, 300 mM NaCl[1], 150 mM imidazole[2], pH 7.4

[1] Unspecific binding of proteins due to electrostatic interactions can be decreased by increasing the NaCl concentration up to 500 mM.

[2] The imidazole concentration required for wash and elution is protein-dependent. Higher or lower concentrations might be needed.

For buffer preparation, please refer to established protocols.

Denaturing protein purification

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 unfolding. On-column refolding of the denatured protein may be possible, but depends on the protein. Advice for overcoming problems associated with inclusion bodies is described in *Section Tips and hints*.

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.

3 Protocols

Sample preparation

For optimal growth and induction, please refer to established protocols.

Cell lysis protocol

1 Dilute the cell paste

- Add 5 to 10 ml of binding buffer for each gram of cell paste.

2 Enzymatic lysis

- Add the following substances to specified final concentrations in the cell suspensions:
  - lysozyme: 0.2 mg/ml
  - DNAse: 20 μg/ml
  - $MgCl_2$: 1 mM
  - Pefabloc™ SC or PMSF: 1 mM
- Stir for 30 min at room temperature or 4°C depending on the sensitivity of the target protein.

If a commercial lysis kit is used; follow the recommended manufacturer's protocol. Note that the protocol may need to be optimized for optimal performance.

3 Mechanical lysis[1]

- Sonication on ice, approx. 10 min, or
- Homogenization with a French press or other homogenizer, or
- Freeze/thaw, repeated at least five times

[1] To prevent clogging of the column/well, mechanical lysis time may have to be extended compared with standard protocols to secure an optimized lysate for sample loading. Also, consider clarification of the sample. Different proteins have different sensitivity to cell lysis and caution has to be taken to avoid frothing and overheating of the sample.

4 Adjust the pH of the lysate:

- Do not use strong bases or acids for pH adjustment (precipitation risk).
- Apply the unclarified lysate on the column/96-well filter plate directly after preparation.

Note: *If the sonicated or homogenized unclarified cell lysate is frozen before use, precipitation and aggregation may increase. New mechanical lysis of the lysate can then prevent increased back pressure problems when loading on the column.*

His GraviTrap TALON protocols

Workmate and LabMate

His GraviTrap TALON columns are delivered in a package that can be converted into a column stand (Workmate) to simplify purification. The plastic tray in the package can be used to collect liquid waste. Connecting LabMate reservoir (Code No. 18-3216-03) to the column increases convenience when handling volumes above 10 ml. This raises the loading capacity to approx. 35 ml in one run.

Purification protocol

1 Prepare and equilibrate

- Cut off the bottom tip, remove the top cap, pour off excess liquid and place the column in the Workmate column stand.
- If needed, mount LabMate on top of the column.
- Equilibrate the column with 10 ml binding buffer. The frits protect the column from running dry during the run.

2 Load sample

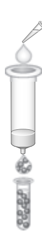
- Add the sample (see *Sample preparation*). A volume of 0.5 to 35 ml is recommended.

  The protein binding capacity of the column is approx. 15 mg histidine-tagged protein/ column (protein-dependent).

Note: *After thorough cell disruption, it is possible to apply the unclarified sample directly without clogging.*

3 Wash

- Wash with 10 ml washing buffer.



4 Elute

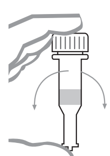
- Apply 3 ml elution buffer and collect the eluate.
- Under denaturing conditions, elute with 2 × 3 ml elution buffer.

Note: *If you use buffers containing denaturing agents or viscous solutions, perform the purification at room temperature.*

His SpinTrap TALON protocols

Purification protocol

- Run purifications on His SpinTrap using a standard microcentrifuge.
- Place the column in a 2 ml microcentrifuge tube to collect the liquid during centrifugation.
- Use a new 2 ml tube for every step.

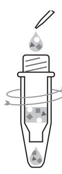


1 Remove storage solution

- Invert and shake the column repeatedly to resuspend the medium.
- Loosen the top cap one-quarter of a turn and break off the bottom closure.
- Place the column in a 2 ml microcentrifuge tube and centrifuge for 30 s at 70 to 100 × *g*.

2 Column equilibration

- Add 600 µl binding buffer.
- Centrifuge for 30 s at 70 to 100 × g.



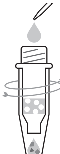
3 Sample application

- Add up to 600 µl sample in one application.

Note: *After thorough cell disruption, it is possible to apply the unclarified sample directly without clogging. However, it is recommended to clarify the sample for optimal recovery.*

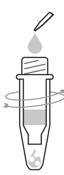
- Seal the column with the top cap and bottom closure and incubate the sample for 5 minutes with slow end-over-end mixing.
- Remove the top cap and bottom closure and centrifuge for 30 s at 70 to 100 × *g*.

Note: *Several sample applications can be performed as long as the capacity of the column is not exceeded.*



4 Wash

- Add 600 µl washing buffer.
- To increase the yield, close the column with the top cap and bottom closure and resuspend.
- Remove the top cap and bottom closure and centrifuge for 30 s at 70 to 100 × *g*.
- Repeat this step once.



5 Elution

- Add 200 µl elution buffer.
- To increase the yield, close the column with the top cap and bottom closure and resuspend.
- Remove the top cap and bottom closure, centrifuge for 30 s at 70 to 100 × *g* and collect the purified sample.
- Repeat this step once.

Note: *The first eluted 200 µl will contain the majority of the target protein.*

His MultiTrap TALON protocols

Purification with His MultiTrap TALON can be performed either by using centrifugation or vacuum pressure.

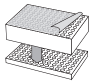
Purification protocol using centrifugation

General considerations

- This protocol is only a general guideline for the purification with His MultiTrap TALON. Optimization may be required depending on source and type of protein.

Purification protocol

1 Remove storage solution

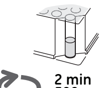


- Remove the bottom seal.
- Gently shake the 96-well filter plate while holding it upside down, to remove any medium stuck on the top seal. Place the plate in upright position.
- Remove the top seal from the plate while holding it against the bench surface.
- Position the plate on top of a collection plate.

Note: *Remember to change or empty the collection plate when necessary during the following steps.*

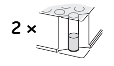
- Centrifuge the plates for 2 minutes at 500 × *g*, to remove the storage solution from the medium.

2 Prewash

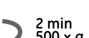


- Add 500 μl deionized water/well.
- Centrifuge for 2 minutes at 500 × g.

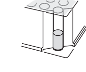
3 Equilibrate



- Add 500 μl binding buffer/well and mix briefly, to equilibrate the medium.
- Centrifuge for 2 minutes at 500 × g.
- Repeat once.



4 Load sample

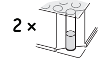


- Apply sample to the wells (maximum 600 µl/well).

Note: *After thorough cell disruption, it is possible to apply the unclarified sample directly without clogging. However, it is recommended to clarify the sample for optimal recovery.*

- Incubate for 3 minutes. (Increase the incubation time if the yield is too low).
- Remove the flowthrough by centrifuging for 4 minutes at 100 × g (or until all wells are empty).

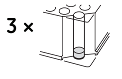
5 Wash



- Add 500 µl washing buffer/well to wash out unbound sample.

- Centrifuge for 2 minutes at 500 × g.
- Repeat once (or until all unbound sample are removed).

6 Elute



- Add 200 µl[1] of elution buffer/well and mix for 1 minute.

- Change collection plate and centrifuge the plates for 2 minutes at 500 × g and collect the fractions.
- Repeat twice (or until all target protein has been eluted).
- 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.)

Vacuum pressure protocol

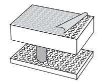
General considerations

- This protocol is only a general guideline for the purification with His MultiTrap TALON. Optimization may be required depending on source and type of protein.
- If problems with cross-contamination due to foaming, poor reproducibility or bubbles in the collection plate occur using vacuum, decrease load of protein (< 0.5 mg 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 be as narrow as possible (not more than 5 mm).
  Use deep round well collection plates (500 µl) to avoid splashes between wells.
  A vacuum pressure of -150 mbar (30 s) followed by -300mbar (<3 s) should be used during elution of purified protein.

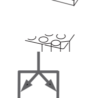
  Note: *Vacuum parameters need to be optimized for each vacuum manifold.*

Purification protocol

1 Remove storage solution



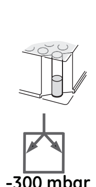
- Remove the bottom seal.
- Gently shake the 96-well filter plate while holding it upside down, to remove any medium stuck on the top seal. Place the plate in upright position.
- Remove the top seal from the plate while holding it against the bench surface.
- Place the 96-well filter plate on the vacuum manifold. Remove the storage solution from the medium by applying a vacuum pressure of -300 mbar for 20 s.



-300 mbar
20 s

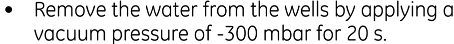
Note: *Position the filter plate on top of a collection plate. Remember to change or empty the collection plate when necessary during the following steps.*

2 Prewash

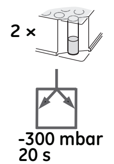


-300 mbar
20 s

- Add 500 µl deionized water/well.

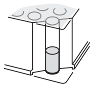


- Remove the water from the wells by applying a vacuum pressure of -300 mbar for 20 s.



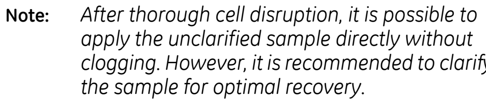
3 Equilibrate

- Add 500 µl binding buffer/well to equilibrate the medium.
- Remove the solution by applying a vacuum pressure of -300 mbar for 20 s.
- Repeat once.

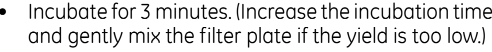


4 Load sample

- Apply sample to the wells (maximum 600 µl/well).



Note: *After thorough cell disruption, it is possible to apply the unclarified sample directly without clogging. However, it is recommended to clarify the sample for optimal recovery.*

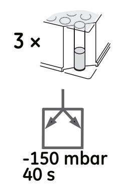


- Incubate for 3 minutes. (Increase the incubation time and gently mix the filter plate if the yield is too low.)

-150 mbar
1 min

Note: *In purifications using robot, the vacuum has to be adjusted to methods applicable to the robot.*

- Remove the flowthrough by applying a vacuum pressure of -150 mbar until all wells are empty (30 to 50 s).



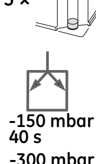
5 Wash

- Add 500 µl washing buffer/well to wash out unbound sample.
- Remove the solution by applying a vacuum pressure of -150 mbar for 30 s.

Note: *-300 mbar for 20 s could also be used if the waste not will be collected.*

- Repeat twice (or until all unbound sample is removed).

6 Elute



- 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 30 s followed by applying a vacuum pressure of -300 mbar for <3 s, or until all droplets under the plate are removed.
- Repeat twice (or until all target protein has been eluted).
- 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.

4 Optimization

Concentration of imidazole

Imidazole at low concentrations is commonly used in the binding and wash buffers to minimize binding of host cell proteins. Imidazole can also be included in the sample (generally at the same concentration as in the wash buffer) to further minimize binding of host cell proteins.

Too high concentration of imidazole in sample and binding buffer may decrease the binding of histidine-tagged proteins. The imidazole concentration must therefore be optimized to ensure the best balance of high purity (low binding of host cell proteins), and high yield (strong binding of histidine-tagged target protein). This optimal concentration is different for different histidine-tagged proteins.

Finding the optimal imidazole concentration for a specific histidine-tagged protein is a trial-and error effort, but 0 to 10 mM in the binding and wash buffers is a good starting point for many proteins. Use high purity imidazole, which gives essentially no absorbance at 280 nm.

5 Tips and hints

The following tips may be of assistance. If you have further questions about His GraviTrap TALON, His SpinTrap TALON or His MultiTrap TALON, please visit www.gelifesciences.com/sampleprep or contact technical support or your local GE Healthcare representative.

Issue	*Possible cause:* Action
1. Flow rate is too slow	*a) Protein is difficult to dissolve or precipitates during purification:* The following additives may be used to solubilize the protein: up to 1% Triton X-100, 1% NP-40, 1% CHAPS, 1.0 M NaCl, 20% glycerol, 10 mM β-mercaptoethanol, 8 M urea, or 6 M Gua-HCl. Mix gently for 30 min to aid solubilization of the tagged protein (inclusion bodies may require longer mixing). Note that Triton X-100 and NP-40 have a high absorbance at 280 nm. Furthermore, detergents cannot be easily removed by buffer exchange. *b) Insoluble protein (inclusion bodies):* The protein can usually be solubilized (and unfolded) from inclusion bodies using common denaturants such as 4 to 6 M Gua-HCl, 4 to 8 M urea (pH 7.4-7.6. Buffers with urea should also include 500 mM NaCl), or strong detergents. Mix gently for 30 min or more to aid solubilization of the tagged protein. Purify in the presence of the denaturant. Use these buffers for sample preparation, as well as binding/wash and elution buffers. To minimize dilution of the sample, solid urea or Gua-HCl can be added. *c) The sample is too viscous:* If the purification has been performed at +4°C, change to room temperature if possible. Increase dilution of the cell paste before lysis or dilute after lysation. Continue lysation until the viscosity is reduced, and/or add an additional dose of DNAse and Mg^{2+}. Filter the sample (or centrifuge if unclarified sample has been used).

Issue	*Possible cause:* Action
2. No, or low yield of histidine-tagged protein in the purified fractions	*a) Elution conditions are too mild (histidine-tagged protein still bound):* Elute with a higher concentration of imidazole in the elution buffer or add EDTA to the elution buffer (will cause stripping of cobalt ions). *b) Histidine-tagged protein not completely eluted:* Elute with a larger volume of elution buffer or add a second elution step. *c) Protein has precipitated in the column/wells:* Decrease amount of sample. Try detergents or change NaCl concentration, or elute under denaturing (unfolding) conditions (use 4 to 8 M urea or 4 to 6 M Gua-HCl). *d) Nonspecific hydrophobic or other interaction:* Add a nonionic detergent to the elution buffer (e.g., 0.2% Triton X-100) or increase the NaCl concentration. *e) Low yield of eluted protein when load of highly unclarifed sample:* Clarify the sample before load on columns or in wells. High level of host proteins and other particles may interfere with the binding of the target protein.

Issue	*Possible cause:* Action
3. Protein found in the flowthrough or wash	*a) Concentration of imidazole in the sample and/or binding buffer is too high:* Decrease imidazole concentration. *b) Incubation time to short:* Increase the incubation time of the sample in columns/wells. *c) 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. *d) Buffer/sample composition is not optimal:* Check pH and composition of sample and binding buffer. Ensure that the concentration of chelating or strong reducing agents in the sample as well as the concentration of imidazole is not too high.

Issue	*Possible cause:* Action
4. The eluted protein is not pure (multiple bands on SDS polyacrylamide gel)	*a) Partial degradation of tagged protein by proteases:* Add protease inhibitors (use EDTA with caution). *b) Contaminants have high affinity for cobalt ions:* Use a higher imidazole concentration in sample and binding buffer to prevent binding of unwanted host cell proteins. 0–10 mM is recommended, but higher concentrations may also be appropriate. Wash thoroughly before elution with wash buffer containing the highest possible imidazole concentration (chosen imidazole concentration must not cause elution of the histidine-tagged protein). *c) Contaminants are associated with tagged proteins:* Add detergent and/or reducing agents before disrupting cells. Increase detergent levels (e.g., up to 1% Triton X-100), or add glycerol (up to 20%) to the wash buffer to disrupt nonspecific interactions. *d) Electrostatic interactions of contaminants with the tagged proteins or the chromatography medium:* Add NaCl up to 500 mM in the buffers. Above 500 mM hydrophobic interactions might occur. *e) Insufficient washing of unbound material:* Add another wash step after sample application to obtain optimal purity.

Issue	*Possible cause:* Action
5. His MultiTrap TALON: Problem with Reproducibility and/or cross contamination caused by foam and/or splash in collection plate when using vacuum	*a) Distance between filter plate and collection plate is too large:* It should not be more than 5 mm between filter plate and collection plate. *b) Solution not aspirated from well:* Increase first elution time at -150 mbar. If that is not working, increase vacuum with caution. *c) Too high vacuum pressure:* Use vacuum pressures between -150 and -200 mbar with a final pressure of -300 mbar in maximum 3 s to remove droplets. If reproducibility not is important remove the last -300 mbar step and gently remove the MultiTrap plate from collection plate to avoid cross contamination. *d) Collection plate is to shallow (<500 μl wells):* Use deep round well collection plate to avoid splashes in to adjacent wells. For recommended collection plate, see *Ordering information*. *e) Medium not completely washed from contamination:* Add more wash steps before eluting the protein. *f) High protein concentration cause foam in collection plate which affect reproducibility:* Decrease load of protein (< 0.5 mg protein bound to medium). If none of the above suggestion works, the recommendation is to change to centrifugation.

6 Ordering Information

Product	Quantity	Code No
His GraviTrap TALON	10 x 1 ml	29-0005-94
His SpinTrap TALON	50 x 100 µl	29-0005-93
His MultiTrap TALON	4 x 96-well plates	29-0005-96

Related product	Quantity	Code No
HiTrap TALON crude	5 × 1 ml	28-9537-66
HiTrap TALON crude	100 × 1 ml[1]	28-9538-05
HiTrap TALON crude	5 × 5 ml	28-9537-67
HiTrap TALON crude	100 × 5 ml[1]	28-9538-09
TALON Superflow	10 ml	28-9574-99
TALON Superflow	50 ml	28-9575-02
His GraviTrap	10 × 1 ml	11-0033-99
His SpinTrap	50 × 100 µl	28-4013-53
His MultiTrap FF	4 × 96-well plates	28-4009-89
His MultiTrap HP	4 × 96 well plates	28-4009-90
Collection plate 500 µl V-bottom	5 × 96-well plates	28-4039-43
PD-10 Desalting Column	30	17-0851-01
PD MidiTrap G-25	50 columns	28-9180-08
PD MiniTrap G-25	50 columns	28-9180-07
PD SpinTrap G-25	50 columns	28-9180-04
PD MultiTrap G-25	4 x 96-well plates	28-9180-06
LabMate PD-10 Buffer Reservoir	10	18-3216-03

[1] Pack size available by special order

Related literature	Code No
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
Protein Sample Preparation Handbook	28-98-87-41

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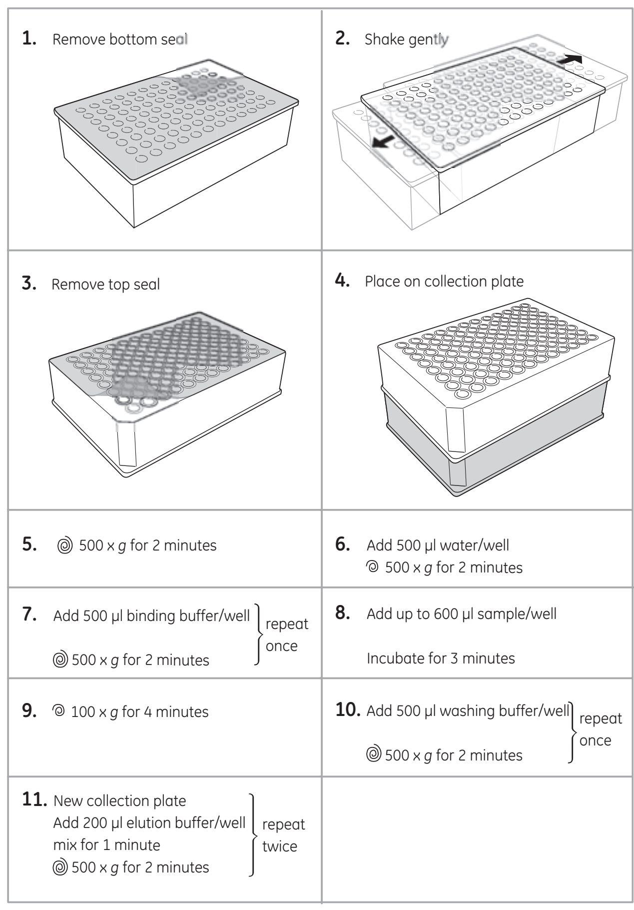
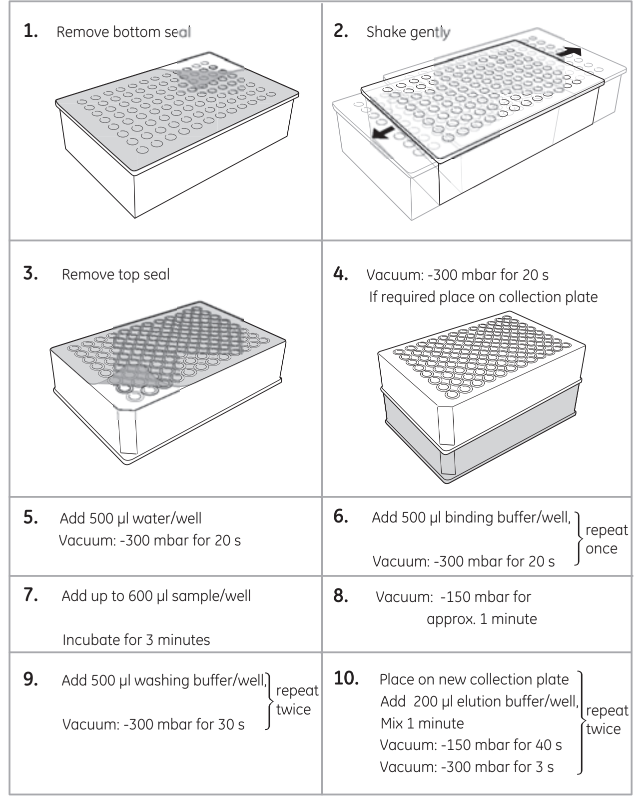
29-0042-12 AB 02/2012

His MultiTrap TALON centrifugation protocol



His MultiTrap TALON vacuum pressure protocol



His Gravitrap TALON protocol

1. Prepare and equilibrate

- Cut off the bottom tip, remove the top cap, pour off excess liquid and place the column in the Workmate column stand.
- Equilibrate the column with 10 ml binding buffer.

2. Load sample

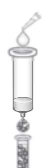
- Add the sample.

A volume of 0.5 to 35 ml is recommended.

3. Wash

- Wash with 10 ml washing buffer.

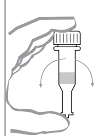
4. Elute



- Apply 3 ml elution buffer and collect the eluate.
- Under denaturing conditions, elute with 2 × 3 ml elution buffer.

His SpinTrap TALON protocol

1. Remove storage solution



- Invert and shake the column repeatedly to resuspend the medium.
- Loosen the top cap one-quarter of a turn and break off the bottom closure.
- Place the column in a 2-ml micro-centrifuge tube and centrifuge for 30 s at 70 to 100 × g.

2. Column equilibration

- Add 600 µl binding buffer.
- Centrifuge for 30 s at 70 to 100 × g.

3. Sample application

- Add up to 600 µl sample in one application.
- End-over-end mixing for 5 minutes.
- Centrifuge for 30 s at 70 to 100 × g.

4. Wash

- Add 600 µl binding buffer.
- Seal the column and resuspend.
- Centrifuge for 30 s at 70 to 100 × g.
- Repeat this step once.

5. Elution

- Add 200 µl elution buffer.
- For a better yeild, seal the column and resuspend.
- Centrifuge for 30 s at 70 to 100 × g and collect the purified sample.
- Repeat once