Instructions for use

# ROTI®Garose
# His/Ni NTA-Beads (0807)
# His/Ni NTA-HPBeads (0805)

Agarose-Beads for one-step isolation of His-tagged proteins by affinity chromatography under low or middle pressure, or also in big volumes or in batch mode. Suitable for denaturing or reducing as well as native conditions. IMAC matrix, nickel- or cobalt charged. NTA ligand.

### I.A. Characteristics

Nickel charged NTA-agarose Beads for affinity chromatography under medium pressure, with high flow rate or with big sample-/matrix volume.
The matrix of ROTI®Garose-His/Ni NTA-Beads or NTA-HPBeads consists of crosslinked and beaded 6 % agarose, NTA-conjugated and charged with divalent Nickel ions. The tetradentate NTA cross-linker binds his-tagged proteins very efficiently, leading to high recovery rates with minimized nickel bleeding into the eluate. Additionally, ROTI®Garose-His/Ni NTA-HPBeads may repeatedly be regenerated, making them very cost-effective.
The Matrix is stable in all commonly used reagents including denaturing reagents (like 8 M urea, 6 M guanidinium hydrochloride) and (dependent on the respective buffer) reducing substances (for instance ≤30 mM Glutathion, ≤10 mM DTT, ≤10 mM DTE, ≤20 mM β-Mercaptoethanol und ≤0,3 % SDS).
In ROTI®Garose-His/Ni NTA-HPBeads, the advantages of the highly efficient His binding have been combined with a bead technology allowing a very rapid flow rate. Those beads are perfectly suitable for MPLC and FPLC, or if His-tag proteins shall be isolated either in particularly short time or in big amounts.

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### I.B. Suitablilty

| | NTA-Beads | NTA-HPBeads |
|---|---|---|
| Batch mode | suitable | suitable |
| Gravity flow | suitable | suitable |
| Spin isolation | suitable | suitable |
| Max. pressure | 1 bar to 500 g | 1 bar to 500 g |
| Max. flow rate | 8 ml/min | >600 cm/h |
| Recommended flow rate | 0,5-1 ml/min | as required |
| Reducing conditions | suitable | suitable |
| Bed volume | small or moderate | as required (also large scale) |
| Concentration | 50 % beads | 50 % beads |
| Preservative | 30 % ethanol | 20 % ethanol |
| Cross-linking | yes | yes |
| Agarose conc. | 6 % | 6 % |
| Ligand | NTA | NTA |
| Charge | $Ni^{2+}$ | $Ni^{2+}$ |

### II. General notes

a) The following protocol has been optimised for the purification of His-tagged proteins under native conditions. In case of denaturing conditions, minor adaptations may be necessary.
b) Determine the amount of resin needed for isolation of the required His-tagged protein (see VI. Buffers and general comments).
c) Application of the buffers and sample has to be performed by pipetting or injection, depending on the column chosen (gravity flow or pressure).
d) In case a gravity flow column is used, cap the column between steps as soon as the last buffer has just run into the surface of the matrix. During application of buffers or sample, make sure to *not disturb* the matrix surface. After application, remove cap in order to run chromatography by gravity. Pouring sample and buffers down a glass rod held against the wall of the column will minimise the introduction of air bubbles. Efficiency of washing may be enhanced by closing the bottom and top of the column and inverting the column in order to disperse the resin.
e) In gravity flow columns, matrix height should not exeed ¼ of the column height. We recommend to de-gas all solutions prior to adding to the column in order to avoid formation of bubbles.
f) Additional equilibration may be omitted if column has been self-packed directly prior to use.
g) Binding capacity of the resin is due to several factors, such as sample concentration, buffer composition, His-tag accessibility etc. An increase in contact time during binding may facilitate binding efficiency.
h) The amount of elution buffer used mainly depends on the protein amount to be eluted and the matrix volume. As a rule of thumb, 1 matrix volume may be applied.
i) We recommend to elute at least 3times and pool the eluates. However, since the yield of eluted proteins decreases with each elution, fractions may be analysed separately regarding the yield, and pooled only if they contain significant amount of His-tagged protein.
j) It is possible that a significant amount of His-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among His-tag proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm) in order to determine the yield of the eluted His-fused protein. In some cases, moderate rolongation of incubation time for elution may increase the elution efficiency.
k) For storage after isolation, it is recommended to remove the imidazole by ultrafiltration or dialysis.
l) In the following protocols, *'1 volume'* always refers to *'volume of matrix'*, which is the *amount of bead suspension* in batch mode, or the *bed volume* for packed columns.
m) For 1 ml gel volume (column bed), 2 ml resuspended agarose beads is necessary.

**III.A. Purification of His-tagged proteins by batch method**

*Elimination of the preservative*

1. Gently shake the bottle of ROTI®Garose-His/Ni NTA-HPBeads to achieve a homogeneous suspension. Immediately pipette the required amount of suspension to an appropriate centrifugation tube.
2. Sediment the beads by centrifugation at 500 x g for 5 mins. and carefully remove (and discart) the supernatant
   *Note:* Alternatively, one may use a bottle top sterile filter for filtration of the NTA-HPBeads. Filter the beads under moderate vacuum and gently transfer the semi dry beads to a suitable container.
3. Add 10 bed volumes of binding buffer (see VII Buffers and general comments). Equilibrate the gel by mixing gently but thoroughly to achieve a homogeneous suspension.
4. Sediment the beads by centrifugation at 500 x g for 5 mins. and carefully remove (and discart) the supernatant.

*Binding of His-tagged proteins*

5. Add the clarified *E. coli* lysate or protein extract. Mix the suspension gently for 30-60 min at room temperature. In some cases, a slight increase of contact time may facilitate binding.
6. Centrifuge the mixture at 500 x g for 5 mins. to sediment the resin and carefully remove the supernatant.
   *Note:* Keep the supernatant at +4 °C until the isolation has been successfully finished.
7. Wash the protein-covered gel beads by adding 10 bed volumes of wash buffer. Mixing gently but thoroughly.
8. Sediment the beads by centrifugation at 500 x g for 5 mins. and carefully remove (and discart) the supernatant.
9. Repeat the washing step twice (total wash 3 x 10 bed volumes of wash buffer).

*Elution of target proteins*

10. Add 1 bed volume of elution buffer to the protein-covered gel beads. Mix gently but thoroughly for 10 mins. at room temperature.
11. Centrifuge the mixture at 500 x g for 5 mins. to sediment the resin and carefully remove the supernatant (eluate 1). Store the eulate at +4 °C.
12. Repeat the elution step twice or more and pool the eluates containing the purified protein.

**III.B. Purification of His-tagged proteins by gravity column chromatography**

*Column packaging*

1. Gently shake the bottle to obtain a homogenous suspension of ROTI®Garose His/Ni NTA-HPBeads resin. Immediately pipette sufficient suspension to an appropriate empty column.
   In case big colums shall be used: Place a funnel in the head of the column and *slowly* run the suspension down the walls of the column. Avoid formation of bubbles.
2. Let the matrix settle. Remove most of the supernatant from the top of the column by pipetting or flow through. Leave 1 cm above the top pf the matrix in order to prevent drying out.
3. Repeat previous steps until the desired column height is obtained, considering the required binding amount and the sample volume.
4. In case the upper end of the column is to be capped (e.g. for storage of the prepacked column), insert the adapter or cap gently in the column head until it begins to displace the liquid. Make sure no air is trapped.
5. Apply distilled water to the column until the column matrix has completely settled and height is constant. In case the desired height is not achieved, add some more material by repeating steps 1 through 4.
6. When the required height of the column matrix is obtained, wash the column with 5 volumes of distilled water in order to completely eliminate the preservative.
7. Equilibrate the column with 5 to 10 volumes of binding buffer (see VI. Buffers and general comments). Cap the column.

*Elimination of preservative*

1. Add 5-10 volumes of binding buffer (see VI. Buffers and general comments) on top of the column. Close the upper and lower end by capping and mix the resin gently by inverting the column.
   *Note:* May be omitted in case freshly packed columns are used.
2. Let the matrix settle, remove the caps and let the buffer flow through.
3. Repeat equilibration steps 2-4 twice.

*Binding of His-tagged proteins*

4. Apply the clarified *E. coli* lysate or protein extract onto the top of the matrix without stirring the surface of the matrix. Let the sample flow into the resin, then close the bottom and the upper cap.
5. Incubate for 30 to 60 mins. Manually mix the resin by inverting the column.
6. Remove both caps and let the sample solution flow through. Keep the flow through at +4 °C until the isolation has been successfully finished.
7. Close the bottom cap, then add 10 bed volumes of washing buffer (see VI. Buffers and general comments). Close the upper cap and mix by inverting the column.
8. Remove the bottom cap and let the wash buffer flow through.
9. Repeat the washing steps 8-9 twice (total wash 3 x 10 bed volumes of wash buffer).
   *Note:* Check the $OD_{280\ nm}$ of the flow through. Wash with wash buffer until this OD reaches the baseline level of the wash buffer.

*Elution of target proteins*

10. Close the bottom cap. Apply 1 bed volume elution buffer to the column (see VI. Buffers and general comments). Close the upper cap and incubate for 10-15 mins. under constant agitation.
    *Note:* Longer incubation time during elution may increase the yield during the first elution round.
11. Let the gel beads sediment.
12. Remove the bottom cap and collect the flow through (eluate) in an appropriate new tube. Store at +4 °C.
13. Repeat the elution steps twice and pool the collected eluates containing the purified protein.

**III.C. Purification of His-tagged proteins by spin isolation (centrifugation)**

Use mini spin columns with inserted frits of 10-20 µm pore size

*Column packaging and equilibration*

1. Gently shake the bottle to obtain a homogenous suspension of ROTI®Garose His/Ni NTA-HPBeads resin. Remove first the upper inlet cap and immediately pipette 100 µl suspension (corresponding to 50 µl gel beads) to an empty spin column.
2. Remove the lower outlet cap and put the spin column into a collecting tube. Centrifuge at 500 x g for 30 seconds.

3. Close the bottom cap and apply 500 µl of binding buffer. Close the upper cap and mix manually by inverting the column.
4. Remove both caps, centrifuge at 500 x g for 30 seconds and discard the flow through.

*Binding of His-tagged proteins*

5. Close the bottom cap. Apply the clarified *E. coli* lysate or protein extract onto the matrix and let the sample mix with the resin.
6. Incubate for 30 to 60 mins. Close the upper cap and mix the resin by inverting the column.
7. Remove both caps and centrifuge at 500 x g for 30 seconds. Keep the flow through at +4 °C until the isolation has been successfully finished.
8. Close the bottom cap, then add 500 µl washing buffer (see VI. Buffers and general comments). Close the upper cap and mix by inverting the column.
9. Remove both caps, centrifuge at 500 x g for 30 seconds and discard the flow through.
10. Repeat the washing steps 8-9 twice (total wash 3 x 500 µl of wash buffer).
    *Note:* Check the $OD_{280\ nm}$ of the flow through. Wash with wash buffer until this OD reaches the baseline level of the wash buffer.

*Elution of target proteins*

11. Close the bottom cap. Apply 500 µl elution buffer to the column (see VI. Buffers and general comments). Close the upper cap and incubate for 10 mins. under constant agitation.
    *Note:* Longer incubation time during elution may increase the yield during the first elution round.
12. Remove both caps and centrifuge at 500 x g for 30 seconds. Collect the flow through (eluate) in an appropriate new tube. Store at +4 °C.
13. Repeat the elution steps twice and pool the collected eluates containing the purified protein.

### III.D. Purification of His-tagged proteins by FPLC

*Column packaging*

1. Gently shake the bottle to obtain a homogenous suspension of ROTI®Garose His/Ni NTA-HPBeads resin. Place a funnel in the head of the column and *slowly* run the suspension down the walls of the column. Avoid formation of bubbles.
2. Let the matrix settle. Remove most of the supernatant from the top of the column by pipetting or flow through. Leave 1 cm above the top pf the matrix in order to prevent drying out.
3. Repeat previous steps until the desired column height is obtained, considering the required binding amount and the sample volume.
4. Insert the adapter or upper cap gently in the column head until it begins to displace the liquid. Make sure no air is trapped.
5. Apply distilled water to the column stream until the column matrix has completely settled and height is constant (corresponding to the height of the column).
6. When the required height of the column matrix is obtained, wash the column with 5 volumes of distilled water in order to completely eliminate the preservative.

*Elimination of preservative*

7. Equilibrate the column with 5 to 10 volumes of binding buffer (see VI. Buffers and general comments).
   *Note:* Check the $OD_{280\ nm}$ of the flow through. Equilibrate with binding buffer until this OD reaches the baseline level of the binding buffer.

*Binding of His-tagged proteins*

8. Apply the clarified (filtred or centrifuged) *E. coli* lysate or protein extract to the column. Use *low* flow rates in order to allow maximal binding of the His-tagged proteins.
   *Note*: Keep the flow through and check whether the protein has been successfully removed from the lysate. In case too much protein is still present, reduce the flow rate further and apply the flow through again to the column.
9. Apply 10 to 20 volumes of washing buffer (see VI. Buffers and general comments)
   *Note:* Check the $OD_{280\ nm}$ of the flow through. Wash with wash buffer until this OD reaches the baseline level of the wash buffer.

*Elution of target proteins*

10. Apply 5 to 10 volumes of elution buffer (see VI. Buffers and general comments) and collect the flow through in fractions. Keep on ice.
11. Check the fractions for presence of the His-tagged proteins (SDS-PAGE, Bradford) and pool the fractions containing the majority of the pure proteins.

*Column flow recommendations*

ROTI®Garose His/Ni NTA-HPBeads are compatible with common low pressure chromatography columns and FPLC™ applications. We recommend columns equipped with an adjustable plunger/flow adapter. Use low rates during loading/binding to allow maximal binding of the His-tagged protein. The flow rate for equilibration, washing and elution may be increased to reduce the purification time.

### IV. Purification of His-tagged proteins under denaturing conditions / Elution from inclusion bodies

Recombinant proteins often form insoluble inclusion bodies. If so these need to be rendered soluble by purification under denaturing conditions, using for example urea or guanidine chloride at relevant stages (see table).
Cells are disrupted under native conditions using lysozyme together with sonication. Following centrifugation, the fused protein is extracted and solubilized from the pellet by using a denaturant agent (urea or guanidine).

*Isolation of inclusion bodies*

1. Thaw the cell pellet from an *E. coli* expression culture on ice (if frozen).
2. Resuspend 1 g of pelleted, wet cells in 5 ml binding buffer on ice (see VI. Buffers and general comments). Pipette up and down, or use stirring until complete resuspension without visible cell aggregates.
3. Add lysozyme to a final concentration of 1 mg/ml. Stir the solution on ice for 30 min.
4. Sonicate the suspension on ice (e.g. 10x 15 sec. bursts with 15 sec. cooling period between). Check samples appearance after sonication. If the lysate is still viscous add 5 µg/ml DNase I and stir on ice for 15 min.
5. Centrifuge the crude lysate at 10,000 x g for 30 mins at +4 ºC to collect the inclusion bodies. Discard supernatant and keep the pellet on ice.

*Solubilization of inclusion bodies*

6. Resuspend the pellet in 10 ml binding buffer (per g wet cells).
7. Centrifuge at 10,000 x g for 30 mins at +4 ºC and discard supernatant.
8. Add 2 ml (per g wet cells) lysis buffer (see VI. Buffers and general comments. Resuspend the cells by vortexing or sonication.

9. Lyze the inclusion bodies by stirring on ice for 60 mins.
10. Centrifuge at 10,000 x g for 30 min at moderate room temperature to eliminate insoluble material, and transfer supernatant to a clean tube.
11. Centrifuge until the supernatant is clear, then freeze. Optionally, filter the supernatant through a 0,45 µm membrane.

*Isolation of Proteins*

Use the instructions given above with respect to the isolation procedure chosen (batch, gravity, spin, FPLC™).
Make sure that all buffers used contain the same denaturing agent as indicated in **VII.B. Buffers for denaturing protein isolation / isolation from inclusion bodies**

## V. Cleaning and Regeneration

In order to preserve the optimum binding capacity during repeated binding and elution cycles, we recommend cleaning and/or regenerating the resin once in a while.
During **cleaning**, contaminants originating from loaded samples are removed like, for instance, unpsecifically bound proteins, precipitated proteins and lipoproteins. This procedure is recommended only if the same recombinant protein is to be isolated.
During **regeneration**, the metal charge and, therefore, the retained protein are completely eliminated. Regeneration frequency cannot be recommended in general, but should be performed if either the binding rate decreases. Regeneration is obligatory, when another protein is to be eluted. It is also recommended, when the coliour of the resin changes due to loss or reduction of nickel ions.
*NOTE:* Resins packed in columns are regenerated in the column by flow through of the regants given below. Resin used in batch mode is regenerated in a suitable container or in spin tubes.

*Cleaning of the resin*

1. Wash the resin with 5 volumes of distilled water.
2. Add 2 to 5 volumes of cleaning buffer to the resin and incubate for 30 mins. to solubilize and desorb residual proteins.
3. Remove the supernatant of cleaning buffer and wash the resin with 10 to 20 bed volumes of distilled water.
4. For direct use: Wash with 10 volumes of binding buffer.
   For storage: Wash with 2 volumes of 30 % ethanol, resuspend the resin in 30 % ethanol and store at +4-8 ºC.

*Regeneration of the resin*

1. Wash the resin with 10 column volumes of distilled water.
2. Remove the metal from the resin by washing with 10 column volumes of regeneration buffer.
3. Wash with 10 volumes of distilled water in order to remove residual EDTA.
4. Add 2 column volumes of 100 mM Nickel aqueous solution (normally chlorides or sulphates are used).
5. Wash with 10 column volumes of distilled water to eliminate the excess of nickel.
6. For direct use: Wash with 10 volumes of binding buffer.
   For storage: Wash with 2 volumes of 30 % ethanol, resuspend the resin in 30 % ethanol and store at +4-8 ºC.

## VI. Buffers and general comments

Determination of the quantity required depends on the amount of His-tagged protein which is to be isolated. The strength of binding of the protein to the resin as well as the yield of His-tag protein will depend on the amino acid composition, the accessibility of the His-tag, 3D structure, molecular weight etc.
As a start one may use a general binding capacity of ca. 50-60 mg/ml gel volume (25-30 mg/ml suspension volume) determined with GFPuv-$(His)_6$

The choice of buffer mainly depends on the particular properties of the protein and has to be optimised for each protein. In general, buffer substances used most frequently are phosphates (10-150 mM). The pH of binding buffers generally leads to neutrality (pH 7.0-8.0), but can vary from 5.5 to 8.5. In order to avoid ionic interchange, add 0.15-0.5 M of NaCl.
Addition of imidazole increases the selectivity of the binding of target protein (10-40 mM). It is important to use high purity imidazole to avoid affecting photometric measurement at $OD_{280\ nm}$.
**Important**: Avoid presence of agents like EDTA or citrate!

For most of the applications it is not necessary to eliminate the His-tag. However, this elimination is necessary for certain applications such as X-ray crystallography or RMN, where the protein structure is to be determined later. For these purposes, make sure your His-tag is spliced to the protein at a protease cleavage site.

### VI.A. Buffers for native protein isolation

*Binding buffer:* A typical binding buffer includes 50 mM $NaH_2PO_4$, 300 mM NaCl, 10 mM imidazole, pH 8.0

*Washing buffer:* 50 mM $NaH_2PO_4$, 300 mM NaCl, 20 mM imidazole, pH 8.0.

*Elution buffer*: 50 mM $NaH_2PO_4$, 300 mM NaCl, 250 mM imidazole, pH 8.0.

### VI.B. Buffers for denaturing protein isolation / isolation from inclusion bodies

Use the same denaturing agent for all buffers involved in one isolation procedure.

*Lysis buffer (for inclusion bodies):* 50 mM $NaH_2PO_4$, 300 mM NaCl, 10 mM imidazole, denaturing agent (e.g. 8 M urea), pH 8.0

*Binding buffer:* 50 mM $NaH_2PO_4$, 300 mM NaCl, 10 mM imidazole, pH of 8.0. (w/o denaturing agent)

*Washing buffer:* 50 mM $NaH_2PO_4$, 300 mM NaCl, 20 mM imidazole, denaturing agent (e.g. 8 M urea), pH 8.0.

*Elution buffer*: 50 mM $NaH_2PO_4$, 300 mM NaCl, 250 mM imidazole, denaturing agent (e.g. 8 M urea), pH 8.0.

### VI.C. Buffers for cleaning and regeneration

*Cleaning buffer:* 0.5 M NaOH
*Regeneration buffer:* EDTA (100 mM), pH 8.0.

## VII. Compatibility of reagents:

| Reagents | Comments |
|---|---|
| ***Buffers*** | |
| Sodium phosphate | Sodium phosphate buffer 50 mM pH 8.0 is recommended. |
| Tris, HEPES, MOPS | Coordinate with metal ions, causing a decrease in binding capacity. Up to 100mM may be used. |
| Sodium Chloride | Avoids unspecific binding (ionic interactions). At least 0.3M should be used. Up to 2 M can be used |
| ***Denaturing Reagents*** | |
| Urea | Solubilizes protein. Use 8 M for purification under denaturing conditions. |
| Guanidine-HCl | Solubilizes protein. Up to 6 M may be used. |
| ***Additives*** | |
| Imidazole | Competes with the His-tag protein. Reduces non specific binding (20 mM). Elute the His-tag protein (up to 100 mM). |
| Glycerol | Avoids hydrophobic interactions between proteins. Up to 50% can be used. |
| EDTA | Coordinates with cations, causing a decreasse in capacity. Not recomended, but up to 1 mM in samples has been used successfully in some cases. |
| Ethanol | Avoids hydrophobic interactions between proteins but may precipitate proteins causing clumn clogging and low flow rates. Up to 20% can be used. |
| ***Reducing Agents*** | |
| Reduced glutathione | Can reduce $Ni^{2+}$ ions at higher concentrations. Up to 30 mM in samples has been used successfully in some cases. |
| ß-Mercapto-ethanol | Avoids formation of disulfide bonds. Can reduce $Ni^{2+}$ ions at higher concentrations. Up to 20 mM in samples has been used successfully in some cases. |
| DTT, DTE | Can reduce $Ni^{2+}$ ions at higher concentrations. Up to 10 mM in samples has been used successfully in some cases. |
| SDS | Avoids hydrophobic interactions between proteins. Coordinates with cations, causing a decreasse in capacity. Not ecommended, but up to 0.3 % in samples has been used successfully in some cases. |
| ***Detergents*** | |
| Non ionic detergents (Tween, Triton, etc.) | Remove background proteins. Up to 2% can be used |

## VIII. Recommended columns

Empty cartridges for protein isolation

In case longer column beds are needed, 2 or 3 cartridges may be interconnected using the Luer adapter (0198.1, 10-32, male/female luer connector), resulting in a matrix of up to 10 cm. Compatible with common chromatography instruments like ÄKTA™FPLC™.

| Art. No. | Volume* | Inner Ø | Frit pore size | Method |
|---|---|---|---|---|
| 1345 | 1 ml | 6,2 mm | 12 µm | FPLC |

* The actual filling volume depends on the respective resin used, varying between approx. 1 and 1.3 ml.


Empty columns for protein isolation

| Art. No. | Type | Fig. | Matrix vol. | Total vol. | Frit pore size | Method |
|---|---|---|---|---|---|---|
| 1541 | Spin | 1 | 100-250 µl | 1,5 ml | 20 µm | Spin |
| 1515 | Grav S | 2 | 100-200 µl | 1 ml | 20 µm | Gravity |
| 1516 | Grav M | 3 | 0,5-2 ml | 12 ml | 20 µm | Gravity |
| 1518 | Grav L | 3 | 2-6 ml | 35 ml | 20 µm | Gravity |
| 1527 | Luer | 4 | 50-100 µl | 0,8 ml | 35 µm | Pressure |
| 1532 | Inject | 5 | 0,8 ml | 0,8 ml | 10 µm | Pressure |

Empty cartridges FPLC

| Art. No. | Type | Vol. (ml)* | Cartri-dges (no.) | Frits (no.) | End caps (no.) | Ø (mm) | Height (mm) |
|---|---|---|---|---|---|---|---|
| 0860 | FPLC-8 | 8 | 3 | 9 | 6 | 12 | 70 |
| 0862 | FPLC-30 | 30 | 2 | 6 | 4 | 21 | 87 |
| 0866 | FPLC-45 | 45 | 2 | 6 | 4 | 21 | 137 |
| 0868 | FPLC-80 | 80 | 1 | 3 | 2 | 26 | 144 |

*packed column matrix

## IX. Trouble Shooting

### IX.A. Sample Application

| Putative cause | Recommendation |
|---|---|
| Sample of high viscosity | |
| Presence of DNA in the sample | Sonify sample until viscosity is reduced or degrade DNA via DNAse |
| Steric hindrance of the substrate | Dilute the sample prior to applica-tion to the column. Purification in batch format may be method of choice. |
| Highly diluted or concentrated sample | |
| Highly diluted sample | Concentrate sample prior to application to the column.<br>Carry out an adsorption step in batch format and pack the column with the pre-adsorbed resin |
| Concentr. sample | Dilute sample prior to loading onto the column |

### IX.B. Adsorption

| Putative cause | Recommendation |
|---|---|
| No binding of target protein to the column | |
| His-tag is not present or has been degraded | Purify protein at lower temperature (4°C). Reduce time needed for purification.<br>Add Protease inhibitors.<br>Double-check recombinant vector for multi-His site. |
| His-tag is not exposed (inaccessible) | Purify in denaturing conditions.<br>Redesign vector in order to change site of the His-tag {N-terminus, C-terminus, or in both positions). |
| Inadequate binding conditions | Check buffers and binding pH.<br>Check reagents used (best before dates). Use fresh chemicals.<br>Reduce imidazole concentration or omit completely in binding buffer.<br>Recheck reagents used in binding buffer for interference with binding reaction.<br>Check whether protein may be found in inclusion bodies (see IV.)<br>Regenerate column/resin |
| Inefficient binding of target protein to the column | |
| Column capacity is exceeded | Apply less protein/sample.<br>Increase matrix volume. |
| Matrix has been used too often | Apply a regeneration step |
| Loss of chelating metal in the resin | Apply a regeneration step.<br>Avoid use and chelating agents. |
| Histidine tail is not well exposed | Apply slower flow rates.<br>Apply or increase incubation time of sample in the column. Invert column after application of sample to disperse the matrix.<br>Choose batch format for binding in order to allow increased contact between resin and rec. protein.<br>Chose buffer with denaturing conditions. |
| Poor protein expression | Optimize bacterial expression conditions. |
| Rec. protein expressed in inclusion bodies. | Modify bacterial growth conditions.<br>Purify under denaturing conditions (see IV.) |
| Matrix bed disturbed (channel-formation) | Re-pack column. |

### IX.C. Elution

| Putative cause | Recommendation |
|---|---|
| High amount of co-eluted proteins (contaminants) | |
| Insufficient washing | Increase volume of washing buffer.<br>Increase number of washing steps.<br>Increase concentration of imidazole in washing buffer.<br>Invert column during washing in order to disperse matrix beads. |
| Inadequate adsorption conditions | Check pH.<br>Add or increase saline concentration in binding buffer in order to avoid non-specific ionic interactions.<br>Add low concentration of non-ionic detergent.<br>Add small quantities of ethylene glycol or glycerol to the binding buffer in order to avoid non-specific hydrophobic interactions.<br>Increase imidazole conc. in binding buffer or apply imidazole gradient (*note:* keep imidazole ≤20 mM). |
| Column / matrix volume too large | Reduce resin quantity. Proteins will compete for less binding sites, increasing binding selectivity.<br>Employ an imidazole concentration gradient to separate the target protein from the rest of retained proteins. |
| Target protein poorly eluted | |
| Too smooth elution conditions | Increase imidazole concentration up to 2.0 M in elution buffer.<br>Increase temperature during elution.<br>Apply slower flow rates.<br>Invert column during elution in order to disperse matrix HPBeads more thoroughly.<br>Apply or increase incubation time after application of sample to the column.<br>Choose batch format for binding in order to allow increased contact between resin and rec. protein. |
| | Change elution conditions / buffer:<br>- Replace imidazole by other chelating reagents (see VI.).<br>- Elute under pH-reduction (pH 4.0) in the presence of imidazole. |

| | |
|---|---|
| | - Elute under denaturing conditions.<br>- Use chelating agent such as EDTA. |
| Recombinant protein precipitates / can be precipitated | Add solubilizing agents.<br>Incubate column with elution buffer for 8-10 h and then elute from the column.<br>Choose batch format in order to reduce local concentration of protein. |
| Elution profile is not reproducible | |
| Sample has been modified (e.g. lost His-tag) | Recheck conditions of bacterial growth / protein expression and sample preparation. Prepare fresh sample, standardise sample preparation.<br>Run the protocol at 2-8 °C.<br>Add protease Inhibitors. |
| Precipitation of proteins or lipids | Regenerate resin/column. |
| PH or ionic forces could have been modified | Prepare new buffers. |
| Loss of binding capacity | Regenerate resin/column. |

**IX.D. Changes of the Resin**

| Putative cause | Recommendation |
|---|---|
| Loss of colour | |
| Chelating reagents present in sample may have caused diminution of metal content | Eliminate chelating reagents in the sample (e.g. by gel filtiation) and regenerate resin/column. |
| Colour shift to brown | |
| Presence of reducing agents in sample | Eliminate reducing agents and regenerate resin/column. |
| Loss of binding capacity | Regenerate resin/column. |

**X. Storage**

Store at +2 to +8 °C. Do not freeze.
HPBeads may be autoclaved at 121 °C for 30 mins.

*For research use only. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical* or in vitro *diagnostics.*

**Hazard and Precautionary Statements**

Please note safety data given on label and MSDS.

- **ROTI®Garose His/Ni NTA-Beads** (Art. No. 0807)
   **Warning** H226-H319-EUH208
- **ROTI®Garose His/Ni NTA-HPBeads** (Art. No. 0805)
   **Warning** H226-H319-EUH208

SDB-Versionen: 08/2021

**ROTI®Garose His/Ni NTA-Beads**

| | |
|---|---|
| **25 ml** | **0807.1** |
| **100 ml** | **0807.2** |

**ROTI®Garose His/Ni NTA-HPBeads**

| | |
|---|---|
| **25 ml** | **0805.1** |
| **100 ml** | **0805.2** |