

Instructions for use



ROTI®Garose His HPBeads plus

Agarose-Beads for one-step isolation of His-tagged proteins by affinity chromatography under low or middle pressure, or also in big volumes, **under presence of EDTA or DTT**

IMAC matrix, nickel-charged. Polychelator ligand. Suitable for LPLC, FPLC and MPLC and all matrix volumes

I. Characteristics

Nickel charged agarose HPBeads with EDTA/DTT compatible polychelator ligand for affinity chromatography under medium pressure, with high flow rate or with big sample-/matrix volume. In ROTI®Garose-His HPBeads plus, 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.

The matrix of ROTI®Garose-His HPBeads plus consists of crosslinked and beaded 6 % agarose, polychelator-conjugated and charged with divalent nickel ions.

The multi dentate cross-linker binds his-tagged proteins very efficiently, leading to high recovery rates with minimized nickel bleeding into the eluate.

The matrix is very stabile and may generally be used with buffers including the following reagents:

≤20 mM EDTA, ≤20 mM DTT, ≤8 M urea, ≤6 M guanidinium hydrochloride, ≤100 % methanol, ≤100 % ethanol, ≤30 % acetonitrile, pH range 4-9.

The resin is adapted to work in native as well as in denaturing conditions.

50 % bead slurry in 20 % ethanol.

I.B. Suitability

	NTA-HPBeads
Batch mode	suitable
Gravity flow	suitable
Spin isolation	suitable
Max. pressure	1 bar to 500 g
Max. flow rate	>600 cm/h
Recommended flow rate	as required
Reducing conditions	suitable
Bed volume	as required (also large scale)
Concentration	50 % beads
Preservative	20 % ethanol
Cross-linking	yes
Agarose conc.	6 %
Ligand	Polychelator
Charge	Ni ²⁺

Important – please note

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.

For 1 ml gel volume (column bed), 2 ml resuspended agarose beads is necessary.

II. General notes

- 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.
- Determine the amount of resin needed for isolation of the required His-tagged protein (see VI. Buffers and general comments).

- Application of the buffers and sample has to be performed by pipetting or injection, depending on the column chosen (gravity flow or pressure).
- 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.
- In gravity flow columns, matrix height should not exceed 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.
- Additional equilibration may be omitted if column has been self-packed directly prior to use.
- 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.
- 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.
- We recommend to elute at least 3 times 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.
- 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 prolongation of incubation time for elution may increase the elution efficiency.
- For storage after isolation, it is recommended to remove the imidazole by ultrafiltration or dialysis.

Carl Roth GmbH + Co. KG

Schoemperlenstraße 3-5 • 76185 Karlsruhe
P.O. Box 100121 • 76231 Karlsruhe
Phone: +49 (0) 721/ 5606-0
Fax: +49 (0) 721/ 5606-149
info@carlroth.com • www.carlroth.com

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The company is a limited partnership with headquarters in Karlsruhe, reg. court Mannheim HRA 100055. Roth Chemie GmbH, with headquarters in Karlsruhe, reg. court Mannheim HRB 100428, is the personally liable partner. Managing Director: André Houdelet. Sales tax identification number: DE 143621073.

- 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 HPBeads plus 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 discard) the supernatant
Note: Alternatively, one may use a bottle top sterile filter for filtration of the HPBeads plus. 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 discard) 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 discard) 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 eluate 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 HPBeads plus resin. Immediately pipette sufficient suspension to an appropriate empty column.
In case big columns 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 of 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 HPBeads plus 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 HPBeads plus 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 of 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 (filtered 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 HPBeads plus 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 and 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 °C 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.
7. Centrifuge at 10,000 x g for 30 mins at +4 °C 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. Lyse 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.

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**

However, in general under extended treatments with **reducing** agents, or when high concentrations of these reagents are used, reduction of the metal ion may result. This will then affect the binding capacity of the resin, so these agents should be avoided.

In these cases we recommend to use

ROTI®Garose His/Ni NTA Beads,

perfectly suited for use under **reducing conditions**.

V. Regeneration:

ROTI®Garose-His HPBeads plus **may not be regenerated**, since they lack stability at pH values of >9.0.

VI. Pretreatment of Ni²⁺-charged resin/columns in particular (optional)

Due to some minor metal bleeding and metal reduction in the charged resin – in most cases after use of reducing reagents in the buffers – a slight brown discoloration may appear. Under most circumstances, this does *not* affect performance.

However, in order to avoid this effect the resin may be pretreated prior to first use, eliminating the *free* metal cations and hence preventing their reduction, subsequent colouring of the resin, and unwanted effects during protein elution.

1. Wash the resin with five volumes of distilled water.
2. Wash the resin with five volumes of binding buffer (without reducing agents in the buffer),
3. Wash the resin with five volumes of elution buffer (without reducing agents in the buffer).
4. Equilibrate with 10 volumes of binding buffer (without reducing agents).

VII. 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, pH, buffers used etc.

As a start one may use a general binding capacity of ca. 80 mg/ml gel volume (40 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 acetate (50 mM) or phosphate (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: HPBeads plus are compatible with EDTA and DTT!

Binding buffer: A typical binding buffer includes 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0

Washing buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 20 mM imidazole, pH 8.0.

Elution buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole, pH 8.0.

In general, there are several ways of protein elution from the columns.

- a) Addition of a competitive ligand (generally imidazole), for instance in the following composition: disodium phosphate (20 mM), NaCl (500 mM), imidazole (500 mM), pH 7.5.

In most cases, 0.5 M of imidazole is enough to efficiently elute the target protein, since most proteins are efficiently eluted at an imidazole concentration of approx. 250 mM. However, in case the elution is not as efficient as required, a concentration gradient of 0-0.5 M, or elution buffer with imidazole of 2.0 M may be applied. Other reagents that can be used as competitive ligands are histidine and ammonium chloride.

Note: Generally, the subsequent elimination of imidazole is not necessary. In case it may hinder downstream processes, it may be eliminated by dialysis, by precipitation with ammonium sulphate or by ultrafiltration using ROTI®Spin centrifugation devices.

- b) Reduction of pH to 3.0-4.0 (with or without gradient)
- c) Elution with EDTA or EGTA (0.05 M). Please note that the latter causes the elution of both the protein and chelating metal.

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.

VIII. Recommended columns



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)*	Cartridges (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



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.

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 application 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. 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. Add Protease inhibitors. Double-check recombinant vector for multi-His site.
His-tag is not exposed (inaccessible)	Purify in denaturing conditions. 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. Check reagents used (best before dates). Use fresh chemicals.

	Reduce imidazole concentration or omit completely in binding buffer. Recheck reagents used in binding buffer for interference with binding reaction. Check whether protein may be found in inclusion bodies (see IV.)
Inefficient binding of target protein to the column	
Column capacity is exceeded	Apply less protein/sample. Increase matrix volume.
Matrix has been used too often	Apply a regeneration step
Loss of chelating metal in the resin	Apply a regeneration step. Avoid use of reducing and chelating agents.
Histidine tail is not well exposed	Apply slower flow rates. Apply or increase incubation time of sample in the column. Invert column after application of sample to disperse the matrix. Choose batch format for binding in order to allow increased contact between resin and rec. protein. Chose buffer with denaturing conditions.
Poor protein expression	Optimize bacterial expression conditions.
Rec. protein expressed in inclusion bodies.	Modify bacterial growth conditions. 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. Increase number of washing steps. Add a bit imidazole (5-10 mM) to buffer used for washing. Invert column during washing in order to disperse matrix HPBeads plus .

Inadequate adsorption conditions	Check pH. Add or increase saline concentration in binding buffer in order to avoid non-specific ionic interactions. Add low concentration of non-ionic detergent. Add small quantities of ethylene glycol or glycerol to the binding buffer in order to avoid non-specific hydrophobic interactions. Increase imidazole conc. in binding buffer or apply imidazole gradient.
Column / matrix volume too large	Reduce resin quantity. Proteins will compete for less binding sites, increasing binding selectivity.
Metal ion chosen is not adequate	Use Cobalt charged resin rather than Nickel charged resin.
Target protein poorly eluted	
Too smooth elution conditions	Increase imidazole concentration up to 2.0 M in elution buffer. Increase temperature during elution. Apply slower flow rates. Invert column during elution in order to disperse matrix HPBeads plus more thoroughly. Apply or increase incubation time after application of sample to the column. Choose batch format for binding in order to allow increased contact between resin and rec. protein.
	Change elution conditions / buffer: - Replace imidazole by other chelating reagents (see VII.). - Elute under pH-reduction (pH 4.0) in the presence of imidazole. - Elute under denaturing conditions. - Use chelating agent such as EDTA.
Recombinant protein precipitates / can be precipitated	Add solubilizing agents. Incubate column with elution buffer for 8-10 h and then elute from the column.

	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. Run the protocol at 2-8 °C. Add protease Inhibitors.
Precipitation of proteins or lipids	Regenerate resin/column.
Buffers have changed	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 filtration) 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.


XI. Storage

Store at +2 to +8 °C. Do not freeze.
HPBeads plus 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.

 **Warning** H226-H319-EUH208

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ROTI®Garose His/Ni HPBeads plus

25 ml	0806.1
100 ml	0806.2