Instructions for use

ROTI®Garose Biotin Beads and Streptavidin Beads

Agarose-Beads for one-step isolation of avidin/streptavidin- or biotin-coupled molecules by affinity chromatography under low pressure.
Streptavidin Beads are also suitable for isolation of purification of proteins via biotinylated antibodies or isolation of iminobiotinylated molecules.

I. Characteristics

Biotin-coupled agarose beads bind with high affinity to avidin and streptavidin, making this resin appropriate for sample isolation as well as removal of avidin or streptavidin from samples. Vice versa, streptavidin agarose beads bind to biotinylated molecules with very high affinity.
Between molecules, with a value of $K_a \sim 10^{-14}$/M, the binding of avidin and streptavidin to biotin is the strongest non-covalent binding known in biochemistry. In both cases, the interaction (biotin-avidin or biotin-streptavidin) is very strong and the bond is stable at extreme pH, organic solvents and denaturing agents.
In biotinylated beads, biotin is immobilised to the beads through a spacer arm of 16 atoms and covalent carboxy/amide linkage. Streptavidin is coupled via an 8 atoms spacer arm.

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This mechanism has been chosen due to its characteristics to not only minimise biotin/streptavidin leakage during elution, but also to enhance the binding capacity by reduction of steric effects.

Merely no unspecific binding of unlabelled molecules takes place, even during purification from raw extracts.
The matrix of Roti®Garose-Biotin- and Streptavidin-Beads consists of beaded 4 % cross-linked agarose.
Elution of biotinylated or avidinated/streptavidinated molecules, respectively, is carried out via 8 M guanidine-HCl or prior to gel loading during heating in gel loading buffer.

Biotin Beads: 50 % bead slurry in water stabilised with 0,02 % sodium azide.
Streptavidin Beads: 50 % bead slurry in 20 % ethanol.

II. General notes

a) The following protocol has been optimised for the purification of avidin/streptavidin- or biotin-tagged molecules under native conditions. However, these are general guidelines. Please optimise for each specific application.
b) Batch purification is the most common way to perform purifications with biotin and streptavidin resins.
c) Determine the amount of resin needed for isolation of the required tagged protein (see VI. Buffers and general comments).
d) Application of the buffers and sample has to be performed by pipetting.
e) 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.
f) 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.
g) Additional equilibration may be omitted if column has been self-packed directly prior to use.
h) The sample has to be dissolved in binding buffer prio to loeading. Optionally, change the buffer system by dialysis or ultrafiltration.
i) Binding capacity of the resin is due to several factors, such as sample concentration, buffer composition etc. Althouogh the binding between avidin/streptavidin and biotin is rapid and strong, an increase in contact time during binding may facilitate binding efficiency.
j) ROTI®Garose Streptavidin-Beads resin may also be used for isolation if iminobiotinylated molecules. In this case please check buffers under VI. Buffers and general comments.
k) 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.
l) In order to optimise the amount of protein isolated, one may elute 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 isolated protein.
m) 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.
n) For 1 ml gel volume (column bed), 2 ml resuspended agarose beads is necessary.

III. Column packaging

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

1. Manually shake the bottle to obtain a homogenous suspension of ROTI®Garose Biotin- or Streptavidin-Beads 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.
   Note: When using **MINI columns** (reaction tube inserts), the matrix may simply be pitetted onto the bottom membrane. After settling of the matrix, proceed at step 7.

2. Let the matrix settle. Decant the resin and discard most of the leftover liquid, leaving 1 cm above the column head to prevent drying out. This is done either by passing it through the column, or pipetting it from the top of the column.
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. Equilibrate the column 3x with 5 volumes of binding buffer (see VI. Buffers and general comments).
   MINI columns: Add 5 vol. binding buffer, cap and invert the column. After settling, remove the supernatant. Cap the column.
   Gravity flow columns: Equilibrate 3 x with flow through of 5 volumes of binding buffer. Cap the column.

IV. Run of the affinity chromatography

The resin may be used with batch methods, gravity flow, and moderate FPLC.
Particular parameters are dependent on the molecules that are to be isolated, may vary from the protocol given here, and should be determined for each assay.

1. Equilibrate the resin or column as given in III.5.
2. Make sure the sample is dissolved in binding buffer. Apply sample onto the top of the matrix without stirring the surface of the matrix. Incubate for 30 mins.
   Note: An increase in contact time may facilitate binding. Binding may also be increased by mixing of the column matrix, for instance by inverting the capped column carefully several times.
3. Remove the lower cap and keep the entire flow through.
4. Wash the column with binding buffer until the $OD_{280\ nm}$ (nearly) reaches the baseline level of the binding buffer (<0.01). Usually, this takes washing with 5-10 volumes. Keep the flow through.
5. Apply elution buffer to the column (see VI. Buffers and general comments) and keep the flow through.
   Note: An increase in contact time may facilitate elution. If the tagged molecules are not eluted directly, incubate for 2 to 10 mins before letting the elution buffer flow through.
   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.

Note: We recommend to dialyze or desalt eluted samples immediately if needed for downstream applications

V. Regeneration:

Since the bond between biotin and streptavidin is very strong, a quantitative removal of the ligand is not possible. Therefore, the beads cannot be successfully regenerated.

VI. Buffers and general comments

Determination of the quantity required depends on the amount of tagged molecule which is to be isolated.
The strength of binding of the protein to the resin as well as the yield of protein will depend on the amino acid composition, the 3D structure, molecular weight, pH, buffers used etc.
As a start one may use a general binding capacity of:
Biotin-Beads: ca. 30 mg/ml gel volume (15 mg/ml suspension volume)
Streptavidin-Beads: ca. 120 nMol/ml gel volume (60 nMol/ml suspension volume). For isolation of biotinylated antibodies by streptavidin beads use approx. 3 mg of biotinylated antibody per ml settled column matrix (6 ml streptavidin agarose bead solution).

Biotin-Beads*:*

Binding buffer:
100 mM NaH_2PO_4, 150 mM NaCl, pH 7.2

Washing buffer: Same as binding buffer

Elution buffer: 8 M guanidine-HCl, pH 1.5
Elution of proteins may also be carried out directly in gel loading buffer during heating prior ot gel loading.

Streptavidin-Beads

*Binding buffer for **biotinylated** molecules:*
20 mM NaH_2PO_4, 150 mM NaCl, pH 7.4

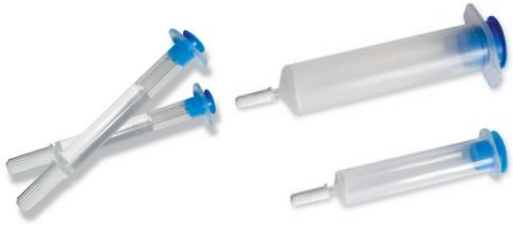
*Binding buffer for **iminobiotinylated** molecules*:
50 mM $(NH_4)_2CO_3$, 0.5 M NaCl, pH 10.0
Washing buffer: Same as binding buffer

*Elution buffer for **biotinylated** molecules:*
8 M guanidine-HCl, pH 1.5

*Elution buffer for **iminobiotinylated** molecules:*
50 mM NH_4Ac, 0.5 M NaCl, pH 4.0

Elution of proteins may also be carried out directly in gel loading buffer during heating prior ot gel loading. Biotinylated antibodies often are not eluted, but rather are directly used for column-based binding of the antigen.

VII. Recommended columns



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Empty columns for protein isolation

Art. No.	Type	Fig.	Matrix vol.	Total vol.	Frit pore size	Method
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

VIII. Trouble Shooting

VIII.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

VIII.B. Adsorption

Putative cause	Recommendation
No binding of target protein to the column	
Inadequate binding conditions	Check buffers and binding pH. Check reagents used (best before dates). Use fresh chemicals. Recheck reagents used in binding buffer for interference with binding reaction. Check whether protein may be found in inclusion bodies.
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
Poor protein expression	Optimize bacterial expression conditions.
Rec. protein expressed in inclusion bodies.	Modify bacterial growth conditions.
Matrix bed disturbed (channel-formation)	Re-pack column.

VIII.C. Elution

Putative cause	Recommendation
High amount of co-eluted proteins (contaminants)	
Insufficient washing	Increase volume of washing buffer. Increase number of washing steps. Invert column during washing in order to disperse matrix Beads.
Inadequate adsorption conditions	Check buffers and binding pH.
Column / matrix volume too large	Reduce resin quantity. Proteins will compete for less binding sites, increasing binding selectivity.
Target protein poorly eluted	
Too smooth elution conditions	Increase temperature moderately during elution. Reduce flow rate. Apply or increase incubation time after application of sample to the column. Invert column during elution incubation in order to disperse matrix Beads. Choose batch format for binding in order to allow increased contact between resin and rec. protein.
Recombinant protein precipitates / can be precipitated	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 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. Storage

Store at +2 to +8 °C. Do not freeze.
Beads 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
P210-P280-P305+P351+P338

ROTI®Garose Biotin Beads	**5 ml**	**0844.1**
	10 ml	**0844.2**
ROTI®Garose Streptavidin Beads		
	5 ml	**0846.1**
	10 ml	**0846.2**