Instructions for use

ROTI®Garose Protein A Beads and Pre-packed Columns

Protein A coated aGarose beads and prepacked columns for low pressure affinity chromatography. High-performance affinity resin for antibody purification.

I.a. Characteristics

The matrix of ROTI®Garose Protein A Beads consists of cross-linked and beaded 4 % aGarose, coated and covalently coupled with protein A from Staphylococcus aureus.
Protein A consists of a single polypeptide chain which contains five highly homologous antibody-binding domains. The binding site is located on the Fe region of the immunoglobulin. The recombinant protein A has high affinity to IgG from a variety of different mammalian species, also binding some populations of IgA and IgM. recProtein A shares IgG binding properties with natural protein A of S. aureus Cowan strain I.
Most immunoglobulins may be eluted in 100 mM glycin or citric acid buffer (pH 3.0).
Column material made from polypropylene and polyethylene (frit). Matrix: Slurry in ethanol (20 %).

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
ip 07/2021

For use in batch mode and gravity flow. May repeatedly be used.
Note: Prepacked columns are filled with preservative (20 % ethanol). Hence, the filling volume is much higher than given in the accompanying product text.

Type	Matrix volume	Total volume	Frit pore size	Binding capacity (human IgG)
MINI	100 µl	1 ml	20 µm	2.5 mg
MIDI	1 ml	12 ml	20 µm	25 mg
MAXI	5 ml	35 ml	20 µm	125 mg

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 pre-packed columns.
Columns are shipped with preservative!

II. Column packaging (only for beads in bulk, not necessary for prepacked columns)

1. Manually shake the bottle to obtain a homogenous suspension of ROTI®Garose Protein A 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.
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. Apply distilled water to the column until 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 V. Buffers and general comments). Cap the column.

Note: 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.

III. Run of the affinity chromatography

Steps 1 and 2 may be omitted if column has been self-packed directly prior to use according to II. Column packaging.

*Note***:** 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.

1. Elimination of preservative
Invert the column, until the resin is completely dispersed. Then remove first the upper, then the lower cap of the column and let the preservative flow from the column by gravity. Apply 5-10 bed volumes of distilled water and let them flow through in order to eliminate the preservative. For batch purification remove the preservative by washing the product on a medium porosity sintered glass funnel.
2. Equilibration of resin/column
Equilibrate the column with 5-10 volumes of binding buffer (see V. Buffers and general comments).
3. Application of sample
Apply sample onto the top of the matrix without stirring the surface of the matrix.
Note: An increase in contact time may facilitate binding. In order to do so, let the sample introduce into the matrix and then cut the flow by capping the lower column end for at least 10 mins.
Sometimes it is advisable to dilute the sample 1:1 with binding buffer before application, in order to maintain ionic strength and pH for optimal binding.
4. Washing of matrix
Wash with 5-10 volumes of binding buffer. A good marker for efficient washing is measurement of the $OD_{280\ nm}$. Washing can be stopped as soon as this OD reaches the baseline level of the binding buffer.
Note: 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.

5. Elution of target protein (pure immunoglobulin)
Apply elution buffer to the column (see V. Buffers and general comments). Mix manually inverting the column. 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 1: In order to enhance elution efficiency, keep elution buffer and resin in contact at least 10 minutes before removing the bottom cap.
Note 2: Add 0.15 ml of buffer pH 9.0 (e.g. Tris 1 M) per ml of purified immunoglobulin in order to neutralize the eluted fractions.

Column flow recommendations (example):
26 cm/h, 0.5-1.0 ml/min, 2.6 psi (0.18 bar)
The resin may be used with batch methods and gravity flow.

IV. Reuse and storage between runs
The column / bead matrix may repeatedly be used, but only for the isolation of similar or closely related immunoglobuline populations.

1. After elution of your required immunoglobuline, make sure the column has been freed of all residual immunoglobulin by elution once again with your chosen elution buffer. Discard the flow through.
   Optionally: Incubate the resin with the elution buffer for 10 mins in the process.
2. Wash repeatedly with distilled water.
3. Wash one with 5 volumes of preservative (20 % highly pure ethanol in water).
4. Add one *column volume* of preservative, cap the column and mix by inverting.
5. Store the column as given in *VII. Storage*.

V. Buffers and general comments

Binding buffer: The typical binding buffer is sodium phosphate (25 mM) or Tris (50 mM), at a pH of 7.0.
Other buffers which may be used are PBS (100 mM), or NaCl (150 mM), at pH 7.2.
Binding occurs through an induced hydrophobic frit and is promoted by addition of salts. At alkaline pH, the interaction between Protein A and antibody is stronger. IgG from most species binds at neutral pH. Binding capacity can be affected by several factors, such as sample concentration, binding buffer or flow rate during sample application.

Elution buffer: Typical elution buffer for immunoglobulins are glycine (100 mM), or citric acid buffer (100 mM), pH 3.0. In general, elution is achieved at reduced pH (3.0 or lower). Depending on the sample, it may be necessary to decrease the pH-value of the elution buffer further below 3.0.
A more drastic method uses potassium isothiocyanate (3 M) as elution buffer.

VI. Trouble Shooting
Please note:
a) Causes and solutions to isolation problems described here are theoretical.
b) The list given below is certainly not depicting all possible explanations and solutions to occuring problems.
In case this trouble shooting section does not fully help, please contact our technical service for advice.

VI.A. Binding efficiency

Putative cause	Recommendation
No binding of target protein to the column	
Conditions in binding or elution have not been optimised.	Optimize pH, flow and temperature, as well as salt or ion concentration.
Channels have formed in column bed so loaded sample runs through column without interacting with Protein A.	Re-pack column.
Column has not been stored in recommended conditions after previous usage.	Follow recommendations given in IV. Reuse and storage between runs.
The antibody/IgG subclass to be purified has low affinity to Protein A.	Check papers published on your particular Ig. Check possible alternatives for purification.
Protease present	Add protease inhibitors to sample loading / wash buffer. Work at lower temperatures (e.g. +4 °C) in order to minimise degradation.

VI.B Elution

Putative cause	Recommendation
Target Ig poorly eluted / degraded	
The antibody is unstable under the conditions chosen for elution	Follow instructions for neutralisation of the eluted fractions (see III.5. *notes*)
Binding efficiency of the Ig to the resin is only poor	See under *VI.A Binding efficiency*
Column flow is very slow	
There are air bubbles in the sample or buffers blocking the flow	Degas sample and buffers used proir to application.
Bubbled formed due to temperature shock during the run	Prior to use, equilibrate the column to the temperature conditions used for the run.

VII. Storage
Store at+2-+8°C. Do not freeze.
Columns and beads may **not** be autoclaved.

For laboratory use only. Not for use in diagnostic in therapeutic procedures.

Hazard and Precautionary Statements
Please note safety data given on label and MSDS.

- **ROTI®Garose Protein A Beads** (Art. No. 1278)
   **Warning** H226-H319
  P210-P280-P305+P351+P338

- **ROTI®Garose Protein A Columns** (Art. No. 4065 / 4066 / 4070)
   **Danger** H225
  P210-P280-P303+P361+P353

ROTI®Garose Protein A Beads	**5 ml**	**1278.1**
	25 ml	**1278.2**
ROTI®Garose Protein A Columns		
MINI	**5x0.1 ml**	**4065.1**
MIDI	**1x1 ml**	**4066.1**
MAXI	**1x5 ml**	**4070.1**