Instructions for use

ROTI®Garose Protein G and A/G HPBeads

Protein G and A/G coated agarose HPBeads for affinity chromatography under medium pressure, with high flow rate or with big sample-/matrix volume. High-performance affinity resin for antibody purification in small-scale on the bench, under medium pressure (MPLC, FPLC), if rapid flow rates are desired or if big samples volumes have to be purified.

I.a. Characteristics

The matrix of ROTI®Garose Protein G and A/G HPBeads consists of cross-linked and beaded 4 % agarose, coated and covalently coupled with protein A from *Staphylococcus aureus* and/or with protein G from *Streptococcus*.
ROTI®Garose Protein A/G HPBeads contain a mixture 50 % ROTI®Garose Protein G and 50 % ROTI®Garose Protein A HPBeads.
Most immunoglobulins may be eluted in 100 mM glycin or citric acid buffer (pH 3.0).
50 % Slurry in ethanol (20 %).
ROTI®Garose Protein G and A/G max be regenerated.

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I.b. Binding parameters

In the recombinant protein G, binding domains specific for albumin as well as cell walls and cell membranes have been removed in the recombinant protein G, in order to ensure the maximum specific IgG binding capacity.
Protein G has high affinity for IgG from a variety of different mammalian species, unlike protein A also binding IgG3 with high affinity. Protein G is also recommended for use in isolation of rat IgGs. Other immunoglobulin populations, like for instance, IgA, IgE or IgM, are bound with particularly low affinity.
The recombinante Protein G produced in *E. coli* has a predicted molecular mass of approximately 21.6 kDa, but migrates with an apparent molecular mass of 32 kDa in SDS-PAGE. The pI is 4.1 and the pH stability 2-10. Optimal binding occurs at pH 5.0, although binding is also good at pH 7.0-7.2.

The recombinant 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. 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.

I.c. Binding affinity[1)]

Protein G / monoclonal antibodies

Human	IgG_1	++++
	IgG_2	++++
	IgG_3	++++
	IgG_4	++++
Mouse	IgG_1	++++
	IgG_{2a}	++++
	IgG_{2b}	+++
	IgG_3	+++
Rat	IgG_1	+
	IgG_{2a}	++++
	IgG_{2b}	++
	IgG_{2c}	++

Protein G / polyclonal antibodies

Chicken	+	Human IgD	+
Cow	++++	Human IgA	+
Horse	++++	Mouse	++
Goat	++	Pig	+++
Guinea pig	++	Rabbit	+++
Human IgG	++++	Rat	++
Human IgM	+	Sheep	++

Protein A/G / mono- and polyclonal* antibodies

Human	IgG_1	Mouse	IgG_1
	IgG_2		IgG_{2a}
	IgG_3		IgG_{2b}
	IgG_4		IgG_3
Rat	IgG_1		IgA
	IgG_{2a}		
	IgG_{2b}	Rabbit*	+++
	IgG_{2c}	Goat*	++

1.d. Binding Capacity

Protein G HPBeads: ca. 20 mg human IgG / ml resin
Protein A/G HPBeads: ca. 25 mg human IgG / ml resin

Important – please note

In the following protocols, *'1 volume'* always refers to *'volume of matrix'*, which is the *amount of bead suspension.*

II. Column packaging

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

1. Manually shake the bottle to obtain a homogenous suspension of ROTI®Garose Protein A or A/G 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. 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 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 V. Buffers and general comments). Cap the column.

Note: 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.

III. Run of the affinity chromatography

*Please note***:**

a) Application of the buffers and sample has to be performed by pipetting or injection, depending on the column chosen (gravity flow or pressure).
b) 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.
c) Binding capacity of the resin is due to several factors, such as sample concentration, buffer composition, flow rate during sample application, sample concentration etc.
d) Steps 1 and 2 may be omitted if column has been self-packed directly prior to use according to II. Column packaging.

1. Elimination of preservative
Apply 5-10 bed volumes of distilled water and let them flow through by gravity or pressure.
2. Equilibration of resin/column
Equilibrate the column with 5-10 volumes of binding buffer at the temperature, at which the isolation will be performed (see V. Buffers and general comments).
3. Application of sample
Apply sample onto the top of the matrix or by injection 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 15 mins.
4. Washing of matrix
Wash with binding buffer until the $OD_{280\ nm}$ reaches the baseline level of the binding buffer. Usually, this takes washing with 5-10 volumes.
5. Elution of immunoglobulins
Apply elution buffer to the column (see V. Buffers and general comments).
Note: The amount of elution buffer used mainly depends on the imunoglubulin amount to be eluted and the matrix volume. As a rule of thumb, 1 matrix volume may be applied.
6. Neutralization of elution buffer (optional)
Add 0.15 ml of neutralisation buffer per ml.

Column flow recommendations:
Recommended maximum flow rate
(100 kPa, 15 cm bed height): 600 cm/h.
Recommended maximum pressure
(15 cm bed height): 300 kPa (3 bar, 40 psi)
The resin may be used with batch methods, gravity flow, and FPLC.

IV. Reuse and storage between runs

The column / bead matrix may repeatedly be used, but only for the isolation of similar or closely related immuno-globuline 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) at a pH of 7.0.
Most IgG populations bind at neutral pH valued.
In some cases, dilution of the sample 1:1 with binding buffer prior to application is advisable in order to maintain the proper ionic strength and pH for optimal binding.

Elution buffer: Typical elution buffer for immunoglobulins are glycine (100 mM), or citric acid buffer (100 mM), pH 3.0-2.5.
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.

Neutralisation buffer (optional): TRIS (1 M), pH 9.0.
Depending on the immunoglobulins isolated and the buffer used, it may me recommended to neutralize the buffer after elution.

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 or G.	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 or G.	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.6)
Binding efficiency of the Ig to the resin is only poor	Check binding parameters under I-b to I.d Check papers published on your particular Ig. Check possible alternatives for purification.
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 to +8 °C. Do not freeze.
Protein A or Protein A/G HPBeads may **not** be autoclaved.

[1)]Harlow, E. And Lane, D. eds. (1988). Antibodies, A. Laboratory Manual. Cold Spring Harbor Laboratory, N.Y., 617-618.

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 Protein G HPBeads** (Art. No. 0808)
   **Warning** H226-H319
  P210-P280-P305+P351+P338

- **ROTI®Garose Protein A/G HPBeads** (Art. No. 0809)
   **Warning** H226-H319
  P210-P280-P305+P351+P338

ROTI®Garose Protein G HPBeads		
	5 ml	**0808.1**
	25 ml	**0808.2**
ROTI®Garose Protein A/G HPBeads		
	0,5 ml	**0809.1**
	1 ml	**0809.2**
	2 ml	**0809.3**