



# GammaBind G Sepharose

## Affinity Chromatography

### Instructions for Use

GammaBind™ G Sepharose™ is GammaBind G, Type 2, covalently immobilized to Sepharose 4B by the cyanogen bromide method. GammaBind G, Type 2, a recombinant form of *streptococcal* Protein G, binds to the Fc region of IgG from a variety of mammalian species. GammaBind G Sepharose may be used to analyze and purify classes, subclasses and fragments of immunoglobulins from any biological fluid or cell culture medium. Since only the Fc region is involved in binding, the Fab region is still available for binding antigen. Hence, GammaBind G Sepharose is very useful for isolation of immune complexes. The potential applications of GammaBind G Sepharose include practically all of the current and projected applications of Protein A Sepharose. However, GammaBind G and Protein A have different IgG binding specificities, depending on the origin of the IgG, see Table 2 Compared to Protein A, GammaBind G binds more strongly to mouse and rat monoclonal IgG and to IgG from goat, sheep, horse, cow, human, rabbit and other mammalian species. GammaBind G will not cross-react with other serum proteins such as IgM, IgE, IgA or transferrin.

GammaBind G, Type 2, Mr 22 000, is produced in *E coli*. and contains two IgG binding regions. The part of the native Protein G molecule that binds albumin has been genetically deleted, thereby avoiding undesirable cross-reactions with albumin.

**Table 1.** Characteristics of GammaBind G Sepharose.

Matrix	4% agarose
Ligand density	Approx. 3 mg GammaBind G, Type 2/ ml drained medium
Coupling chemistry	cyanogen bromide method
Average particle size	90 µm
Dynamic binding capacity <sup>1</sup>	18 mg human IgG/ml drained medium 6 mg mouse IgG/ml drained medium
Maximum operating backpressure	0.008 MPa (0.08 bar, 0.9 psi)
pH stability <sup>2</sup>	
Working range	3 to 9
Cleaning-in-place	2 to 9
Chemical stability:	Stable to all commonly used aqueous buffers and additives such as 1 M acetic acid, 1% SDS and 6 M guanidine hydrochloride.
Physical stability	Negligible volume variation due to changes in pH or ionic strength
Storage	2°C to 8°C in 20% ethanol

<sup>1</sup> The binding capacity was estimated under the following conditions:  
Binding buffer: 0.01 M phosphate sodium buffer, 0.15 M NaCl, 0.01 M EDTA pH 7.0 Elution buffer: 0.5 M acetic acid pH 3.0.

<sup>2</sup> Working range: pH interval where the medium can be operated without significant change in function.  
Cleaning-in-place: pH stability where the medium can be subjected to cleaning- or sanitization-in-place without significant change in function.

**Table 2.** The relative binding strength of polyclonal IgG from various species to GammaBind G and Protein A.

<b>Species</b>	<b>GammaBind G</b>	<b>Protein A</b>
Rat	+	-
Goat	++	-
Sheep	++	-
Cow	++	-
Horse	++	-
Human	++	++
Rabbit	++	++
Mouse	++	+
Guinea Pig	++	++

++ = strong binding

+ = intermediate binding

- = weak or no binding

# 1 Preparing the medium

GammaBind G Sepharose is supplied preswollen in phosphate buffered saline (PBS), pH 7.0 containing 20% ethanol as a preservative. Prepare a slurry by decanting the phosphate buffered saline solution and replace it with binding buffer, see below, in a ratio of 75% settled medium to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rate after packing is completed.

For batch procedures remove the phosphate buffered saline solution by washing the medium on a sintered glass filter (porosity G3).

## 2 Packing Sepharose 4B

- 1 Equilibrate all material to the temperature at which the chromatography will be performed.
- 2 De-gas the medium slurry.
- 3 Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
- 4 Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
- 5 Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump, or an ÄKTA™ chromatography system.
- 6 Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. Do not exceed 75% the maximum pressure given in Table 1.

**Note:** *If you have packed at the maximum linear flow velocity, do not exceed 75% of this in subsequent chromatographic procedures.*

- 7 Maintain the packing flow rate for 3 column volumes after a constant bed height is reached.

### Using an adapter

Adapters should be fitted as follows:

- 1 After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
- 2 Insert the adapter at an angle into the column, making sure that no air is trapped under the net.
- 3 Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.

- 4 Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to make sure that air is removed.
- 5 Lock the adapter in position, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adapter on the medium surface as necessary.

## 3 Binding

IgG from most species binds to GammaBind Plus Sepharose at IgG from most species binds GammaBind G Sepharose at neutral pH and physiological ionic strength.

As a general method we recommend 0.01 M sodium phosphate, 0.15 M NaCl, 0.01 M EDTA, pH 7.0 as binding buffer.

Adjust pH of the sample before it is applied to the column either by buffer exchange on a HiTrap™ Desalting column, PD-10 column or a HiPrep™ 26/10 Desalting column depending on the sample volume.

The binding capacity of GammaBind G Sepharose depends on the source of the particular immunoglobulin, see Table 3. However, the total capacity depends upon several factors, such as the flow rate during sample application, the sample concentration and binding buffer. Table 3 shows the total capacity under defined conditions for IgG from some species.

**Table 3.** The total IgG capacity of GammaBind G Sepharose under defined conditions<sup>1</sup>, for various species.

Species	Total IgG capacity (mg/ml drained medium)
Human	18
Rat	7
Rabbit	19
Goat	19
Cattle	16
Mouse	10

<sup>1</sup> Binding buffer used was 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.0 and elution buffer used was 0.5 M ammonium acetate pH 3.0.

**Note:** *The binding capacity values listed above are typical for the given species. However, there might be considerable deviations in binding capacity for different immunoglobulins derived from the same species, even if they are of the same subclass.*

## 4 Elution

Bound antibodies can be eluted with high yields over a pH range from 2.5 to 3.0.

As a general method, we recommend 0.5 M acetic acid adjusted to pH 3.0 with ammonium hydroxide.

As a safety measure to preserve the activity of acid labile IgG's, we recommend adding 60 to 200 µl of 1 M Tris-HCl, pH 9.0 per ml eluted fraction, to neutralize the eluted antibodies.

## 5 Regeneration

After elution, the medium should be washed with 2 to 3 column volumes of cleaning buffer, 1 M acetic acid, pH 2.5, followed by re-equilibration with 2 to 3 column volumes of binding buffer.

## 6 Storage

For longer periods of storage, keep GammaBind G Sepharose at 2°C to 8°C in a suitable bacteriostat, e.g., 20% ethanol. The chromatography medium must not be frozen.

## 7 More information

Please visit [www.cytiva.com/protein-purification](http://www.cytiva.com/protein-purification) or contact our technical support or your local representative.

## 8 Ordering information

Product	Quantity	Code No
GammaBind G Sepharose	5 ml	17088501
	25 ml	17088502
	250 ml	17088504

Related products	Quantity	Code No
HiPrep 26/10 Desalting	1 × 53 ml	17508701
HiPrep 26/10 Desalting	4 × 53 ml	17508702
HiTrap Desalting	5 × 5 ml	17140801
PD-10 Desalting Column	30	17085101

Literature	Quantity	Code No
Antibody Purification Handbook	1	181037-46
Affinity Chromatography Handbook, Principles and Methods	1	18102229
Affinity Chromatography Columns and Media Selection Guide	1	18112186



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