GammaBind G Sepharose

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 F_c 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 F_c region is involved in binding, the F_{ab} region is still available for binding antigen. Hence, GammaBind G Sepharose is extremely 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.

Sepharose

GammaBind G, Type 2, M_r 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. Medium characteristics

Ligand density:	~3 mg GammaBind G, Type 2/ml drained medium
Available capacity*:	18 mg human IgG/ml drained medium
	6 mg mouse IgG/ml drained medium
Bead structure:	4% agarose
Bead size range:	45–165 μm
Mean bead size:	90 μm
Max. operating backpressure:	0.008 MPa (0.08 bar, 0.9 psi)
pH stability**	
Long term:	3–9
Short term:	2–9
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.

* 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.

** The ranges given are estimates based on our knowledge and experience. Please note the following:
i) pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
ii) pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

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

Species	GammaBind G	Protein A
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.
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 rate, do not exceed 75% of this in subsequent chromatographic procedures.
7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adaptor

Adaptors 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 adaptor at an angle into the column, ensuring 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 ensure that air is removed.

5. Lock the adaptor 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 adaptor on the medium surface as necessary.

3. Binding

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, PD-10 or HiPrep™ 26/10 Desalting 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*, for various species.

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

* 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 60–200 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 2–3 bed volumes of cleaning buffer, 1 M acetic acid, pH 2.5, followed by re-equilibration with 2–3 bed volumes of binding buffer.

6. Storage

For longer periods of storage, keep GammaBind G Sepharose at 4–8 °C in a suitable bacteriostat, e.g 20% ethanol. The chromatography medium must not be frozen.

7. Further information

Check www.gelifesciences.com/protein-purification for more information. Useful information is also available in the selected handbooks, see ordering information.

8. Ordering information

Product	Pack size	Code No.
GammaBind G Sepharose	5 ml	17-0885-01
GammaBind G Sepharose	25 ml	17-0885-02

Related products	Pack size	Code No.
HiTrap Desalting	5×5 ml	17-1408-01
HiPrep 26/10 Desalting	1	17-5087-01
PD-10 Desalting columns	30	17-0851-01

Literature		
Antibody Purification Handbook	1	18-1037-46
Affinity Chromatography Handbook, Priciples and Methods	1	18-1022-29
Affinity Chormatography, Column and Media Guide	1	18-1121-86

www.gelifesciences.com/protein-pruification

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare UK Ltd
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue
P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

GammaBind, HiTrap, HiPrep, Sepharose and Drop Design are trademarks of GE Healthcare Companies. GE, imagination at work, and GE monogram are trademarks of General Electric Companies.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. GE Healthcare reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation. Contact your local GE Healthcare representative for the most current information.

GE Healthcare Bio-Sciences AB, a General Electric Company.

© 2006 General Electric Company – All rights reserved.

imagination at work

Elanders 2009

71-7057-00 AH