GE Healthcare
Life Sciences

Instructions 71-7058-00 AI **Affinity Chromatography**

GammaBind™ Plus Sepharose™

GammaBind™ Plus Sepharose is GammaBind G, Type 3, covalently immobilized to Sepharose CL-6B by malimide linkage. This rigid matrix results in easy handling and fast separations.

GammaBind G, Type 3, a recombinant form of *streptococcal* protein G, binds to the Fc region of IgG from a variety of mammalian species. GammaBind Plus 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 Plus Sepharose is very useful for isolation of immune complexes.

The potential applications of GammaBind Plus include practically all of the current and projected applications of protein A. However, GammaBind Plus and protein A have different IgG binding specificities, depending on the origin of the IgG. Compared to protein A, GammaBind Plus binds more strongly to mouse and rat monoclonal IgG and to IgG from goat, sheep, horse, cow, human, rabbit and other mammalian species. GammaBind Plus will not cross-react with other serum proteins such as IgM, IgE, IgA or transferrin.

GammaBind G, Type 3, Mr 15 000, 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 Plus Sepharose.

Matrix	6% cross-linked agarose
Ligand density	Approx. 3 mg GammaBind G, Type 3/ ml drained medium
Coupling chemistry	malimide linkage
Average particle size	90 µm
Dynamic binding capacity[1]	35 mg human IgG/ml drained medium 7 mg mouse IgG/ml drained medium
Maximum operational velocity	130 cm/h
Maximum operating backpressure	0.015 MPa (0.15 bar, 2 psi
pH stability[2]	
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
Sanitization:	Sanitize the column with 70% ethanol
Storage	2ºC to 8ºC in 20% ethanol

[1] The binding capacity was estimated in following conditions:
Binding buffer: 0.01 M sodium phosphate buffer,
0.15 M NaCl, 0.01 M EDTA, pH 7.0.
Elution buffer: 0.5 M acetic acid, pH 3.0.

[2] 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.

1 Preparing the medium

GammaBind Plus Sepharose is supplied preswollen in phosphate buffered saline (PBS), pH 7.0 containing 20% ethanol as 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 medium porosity sintered glass funnel.

2 Packing media based on Sepharose CL-6B

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. However, the maximum flow velocity, see Table 1, is typically employed during packing. Do not exceed the maximum backpressure given in Table 1.

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

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

3 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, 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, or an ÄKTA™ chromatography system,

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 adapter in position, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the medium bed is stable. Re-position the adapter on the medium surface as necessary.

4 Binding

IgG from most species binds to GammaBind Plus 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 the pH of the sample before it is applied to the column, either by buffer exchange on a HiTrap™ Desalting column, PD-10

column or HiPrep™ 26/10 Desalting column depending on the sample volume.

The binding capacity of GammaBind Plus Sepharose depends on the source of the particular immunoglobulin, see Table 2. However, the total capacity depends upon several factors, such as the flow rate during sample application, the sample concentration and binding buffer. Table 2 shows the total capacity under defined conditions for IgG from some species.

Table 2. The total IgG capacity of GammaBind Plus Sepharose under defined conditions[1], for various species..

Species	Total IgG capacity (mg/ml drained medium)
Hamster	14
Rat	15
Mouse	18
Rabbit	26
Goat	27
Human	27
Sheep	27
Horse	26

[1] The total capacity was determined under conditions of saturation with at least a two-fold excess of serum. Binding buffer used was 0.01 M sodium phosphate, 0.15 M NaCl, 0.01 EDTA, pH 7.0 and elution buffer used was 0.5 M acetic acid, 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.*

5 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 the addition of 60 to 200 µl 1 M Tris-HCl, pH 9.0 per ml eluted fraction, for neutralization of the eluted fractions.

6 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.

7 Storage

For longer periods of storage, keep the medium at 2°C to 8°C in a suitable bacteriostat, e.g., 20% ethanol.

8 More information

Please visit www.gelifesciences.com/protein-purification or contact our technical support or your local representative.

9 Ordering information

Product	Quantity	Code No
GammaBind Plus Sepharose	5 ml	17-0886-01
	25 ml	17-0886-02
	500 ml	17-0886-04

Related products	Quantity	Code No
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
HiTrap Desalting	5 × 5 ml	17-1408-01
PD-10 Desalting Column	30	17-0851-01

Literature	Quantity	Code No
Antibody Purification Handbook	1	18-1037-46
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Chromatography Columns and Media Selection Guide	1	18-1121-86

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