

ProductInformation

PROTEIN G IMMOBILIZED ON AGAROSE

SIGMA PRODUCT NUMBERS:

P-7700 Protein G on cross-linked 4% beaded agarose
 P-4691 Protein G on cross-linked 4% beaded agarose, fast flow
 P-3296 Protein G on Sepharose® 4B fast flow
 5-4840 HiTrap® Protein G column, Sepharose HP, 1 ml
 5-4841 HiTrap Protein G column, Sepharose HP, 5 ml
 5-4842 MAbTrap® G-II Kit, Sepharose HP, 1 ml

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PHYSICAL PROPERTIES:

Appearance: P-7700 is a lyophilized powder stabilized with lactose. P-4691 is a suspension in 20% ethanol containing 0.5M NaCl. P-3296 is a suspension in 20% ethanol. The HiTrap products are ready-to-use, disposable columns in a polypropylene housing with porous top and bottom column frits and all necessary fittings. The resin is in 20% ethanol. The MAbTrap kit contains one 1 ml HiTrap Protein G column with all necessary fittings, 50 ml of binding buffer (10X concentration), 15 ml of elution buffer (10X concentration) and 25 ml of neutralizing buffer.

Average particle size: cross-linked 4% beaded agarose, cross-linked 4% beaded agarose fast flow, and Sepharose 4B fast flow are 45-165 µm; Sepharose HP is approximately 34 µm.⁶

Molecular weight of Protein G: Approximately 20,000 d⁴
 Isoelectric point (pI) of Protein G: 4.1⁶ or 4.19⁴
 Number of IgG binding sites per Protein G molecule: 2^{4,6}

PRODUCT NO.	ACTIVATION ^a	SPACER ATOMS	LIGAND ^b mg/ml	CAPACITY ^c
P7700	cyanogen bromide	1		>20
P4691	cyanogen bromide	1	approx 4	Min. 20
P3296	cyanogen bromide	1	approx 2	>20
5-4840	N-hydroxysuccinimide	9	2	approx 25
5-4841	N-hydroxysuccinimide	9	2	approx 25
5-4842	N-hydroxysuccinimide	9	2	approx 25

- a - Ligands are covalently attached to agarose by the N-hydroxy succinimide activation method⁶ or by cyanogen bromide
 b - mg of Protein G immobilized per ml of packed resin
 c - mg of human IgG bound per ml of packed resin

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METHOD OF PREPARATION:

Protein G is a cell wall protein (originally isolated from Type G Streptococci) that binds the Fc region of immunoglobulin G (IgG) with high affinity. In addition to the Fc receptor, intact Protein G has membrane spanning regions as well as specific binding sites for albumin and for the Fab region of immunoglobins.^{1,2,3} P-7700 and P-4691 are prepared from recombinant Protein G', which is a truncated protein that retains only the Fc binding sites, i.e. has no albumin binding sites.⁴ The other resins are prepared from a recombinant Protein G produced by Pharmacia, Inc., that retains the two high affinity Fc binding sites but also binds the Fab region with low affinity.⁶ Protein G is bound to the agarose bead through the amino terminus and ϵ -amino groups on lysine.

STABILITY / STORAGE SUPPLIED:

The lyophilized resin, P-7700, can be stored frozen at -20°C . The hydrated resins and the ready-to-use columns should be stored refrigerated. To prevent bacterial growth in the hydrated resin, a bacteriostat, such as 20% ethanol, 0.02% sodium azide, or 0.02% thimerosal, should be included in the storage buffer. The resins cannot be autoclaved. Do not freeze the resin after it has been hydrated, since the beads will crack and lose binding capacity.

Protein G immobilized on agarose is stable to all commonly used aqueous buffers. The working pH range of the resin is 3-9 (long-term) or 2-10 (short term). It is also stable in the following solutions: 70% ethanol, 1 M acetic acid, 5 M NaCl, 1% SDS, 3 M isothiocyanate, 6 M guanidine HCl (7 days at 37°C), 0.1 M glycine NaOH, pH 11 (2 hr at room temperature), 1 M HCl (2 hr at room temperature), and 8 M urea (2 hr at room temperature).⁶

USAGE:

Resin preparation: P-7700 is hydrated by placing the resin in excess water, approximately 25 ml per g of resin, for 30 min. One gram of dry beads will give 4-5 ml of packed resin. The lactose stabilizer, ethanol, or excess NaCl should be removed from P-7700, P-4691, and P-3296 prior to use. If the resin will be used in immunoprecipitation, thoroughly wash the resin on a Buchner funnel with gentle vacuum using approximately 50 ml deionized water per g of resin. Do not allow the resin to dry. Resuspend the washed resin with excess working buffer prior to adding it to the samples. If the resin is to be used in a column, pour off the excess water or storage medium, and resuspend the resin in excess starting buffer or 20 mM sodium phosphate buffer, pH 7.0. Pack the column. Equilibrate the column bed with 5 column volumes of starting buffer. This will effectively remove residual bacteriostats and stabilizers from the resin.

To prepare the HiTrap columns for use, remove the ethanol with three column volumes of deionized water or 20 mM sodium phosphate buffer, pH 7.0, then equilibrate the column with 2 column volumes of starting buffer.

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USAGE: (continued)

Do not allow the column to run dry since that will cause channeling of the resin bed.

Sample preparation: Protein G binds IgG at neutral to basic pH. The sample matrix can be serum, ascites fluid, or any low ionic strength neutral buffer, such as 20 mM sodium phosphate buffer, pH 7.0. The pH of the samples should be the same as that of the starting buffer. For affinity chromatography, clarify samples that are cloudy or have particulate matter by filtration or by centrifugation.

IgG purification by affinity chromatography: Load the sample onto the column. When the fluid layer reaches the top of the resin bed, wash with 1-2 column volumes of starting buffer or 20 mM sodium phosphate buffer, pH 7.0. If the initial column effluent contains appreciable IgG and the column capacity has not been exceeded, the effluent can be recycled through the column at a lower flow rate to enhance IgG removal. Wash the column with an additional 3-4 column volumes of starting buffer or 20 mM sodium phosphate buffer, pH 7.0. Elute IgG with 1-3 column volumes of 100 mM glycine HCl, pH 2.7. Bring the fractions containing IgG to neutral pH with 1 M tris HCl, pH 9.0 or 0.1 N NaOH. Reequilibrate the resin with a neutral buffer.

Slow flow rates give maximal binding and recovery of IgG. Columns of 4% beaded agarose fast flow, and Sepharose 4B fast flow can be run at 0.8 ml/min. Columns prepared from 4% cross-linked beaded agarose should be run at about 0.2 ml/min. The maximum flow rate for HiTrap columns is 4 ml/min.⁶

IgG purification by immunoprecipitation: Add an appropriate volume of washed resin to the sample. Mix the sample and resin on a rocking or oscillatory shaker for 1 hr. Collect the resin on a Buchner funnel under gentle vacuum, and wash the resin with ten resin volumes of working buffer or 20 mM sodium phosphate buffer, pH 7.0. Resuspend the resin in 2-3 resin volumes of acidic buffer, such as 100 mM glycine HCl buffer, pH 2.7. Mix on a rocking or oscillatory shaker for 15 minutes to elute the IgG. Collect the eluate by vacuum filtration or by centrifugation. Bring the eluate to neutral pH with 1 M tris HCl, pH 9, or 0.1 N NaOH. If the resin is to be reused, equilibrate it with a neutral buffer.

Cleaning the resin: The resin does not need to be cleaned after each use. However, if serum or ascites fluid has been run on the resin, it can be sanitized with 70% ethanol. If the binding capacity of the resin is reduced, it may be restored by removing non-specifically bound proteins. Generally, these proteins will elute in 1-5 M NaCl solution, 100 mM tris or borate buffer, pH 8.5, containing 0.5 M NaCl, or 100 mM sodium acetate buffer, pH 4.0, containing 0.5 M NaCl. If this does not restore binding capacity, any of the following solutions can be used: 0.1 N HCl; 100 mM glycine NaOH buffer, pH 11; 6 M urea; 8 M guanidine HCl, or 1% SDS.⁶ Reequilibrate the resin with 4-5 column volumes of a neutral buffer. If the resin is to be stored, add a bacteriostat to the final buffer wash. Note that urea, guanidine HCl, and surfactants will denature Protein G, and complete renaturation may not occur during reequilibration.

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REFERENCES:

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3. Bjorck, L., and Kronvall, G., *J. Immunol.* 133, 969-974, (1984).
4. Goward, C.R., et al., *Biochem. J.* 267, 171-177, (1990).
5. Guss, B., et al., *EMBO J.* 5, 1567-1575, (1986).
6. Pharmacia Technical Literature.

COMPARISON OF IgG BINDING BY PROTEIN G AND PROTEIN A

Species	Protein G	Protein A
Monoclonal: ³		
Human IgG ₁	High	high
Human IgG ₂	High	high
Human IgG ₃	High	--
Human IgG ₄	High	high
Mouse IgG ₁	High	low
Mouse IgG _{2a}	High	high
Mouse IgG _{2b}	High	high
Mouse IgG ₃	High	low
Rat IgG ₁	Low	low
Rat IgG _{2a}	High	--
Rat IgG _{2b}	Low	--
Rat IgG _{2c}	Low	low
Polyclonal IgG: ^{5,6}		
Bovine	High	low
Chicken	--	--
Dog	Low	high
Goat	High	low
Guinea Pig	Low	high
Horse	High	--
Human	High	high
Mouse	Low	low
Porcine	High	high
Rabbit	High	high
Rat	High	--
Sheep	High	--