83219 Protein G, immobilized on cross-linked 4% agarose

PHYSICAL PROPERTIES:

Lyophilized powder stabilized with lactose.

Average particle size:	45-165 μm
Molecular weight of Protein G:	Approximately 20,000 d [4]
Isoelectric point (pI) of Protein G:	4.16 or 4.194
Number of IgG binding sites per Protein G molecule:	2 [4,6]
Activation:	Ligands are covalently attached to agaraose by cyanogen bromide
Spacer Atoms:	1
Ligand (mg of Protein G immobilized per ml of packed resin):	not available
Capicity (mg of human IgG bound per ml of packed resin):	>20

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]. 83219 is 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] Protein G is bound to the agarose bead through the amino terminus and ε-amino groups on lysine.

STABILITY / STORAGE SUPPLIED:

The lyophilized resin, 83219, can be stored frozen at -20°C. The hydrated resin should be stored refrigerated. To prevent bacterial growth in the hydrated resin, an antibacterial agent, 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:

83219 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 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. 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 prepared from 4% cross-linked beaded agarose should be run at about 0.2 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.

REFERENCES:

1. Akerstrom, B., and Bjorck, L., *J. Biol. Chem.* 261, 10240-10247, 1986.
2. Akerstrom, B., et al., *J. Immunol.* 135, 2589-2592, (1985).
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.

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.