Protein G Resin

Technical Manual No. 0209 **Version 20060712**

I. Description

Protein G Resin (Cat. No. L00209) is useful for purification and isolation of IgG. Protein G, a bacterial cell wall protein isolated from group G *Streptococci*, binds to mammalian IgGs mainly through Fc regions. Native Protein G has 3 IgG binding domains and also sites for albumin and cell-surface binding. Albumin and cell-surface binding domains have been eliminated from recombinant Protein G to reduce nonspecific binding. Additionally, 3×Cys tag was engineered to the C-terminal of rec-protein G to facilitate its immobilization. Although the tertiary structures of Protein A and Protein G are very similar, their amino acid compositions differ significantly, resulting in different binding characteristics. Protein G may be used for purification of mammalian monoclonal and polyclonal IgGs that do not bind well to Protein A. Protein G has greater affinity than Protein A for most mammalian IgGs, especially for certain subclasses including human IgG3, mouse IgG1 and rat IgG2a. Unlike Protein A, Protein G does not bind to human IgM, IgD and IgA.

II. High lights

- Broad IgG binding spectrum
- Binding specificity complements the different Protein A
- Agarose media
- No specific albumin binding
- Optimized homogeneous recombinant ligand
- High capacity

III. Characteristics of Protein G Resin

Ligand	Recombinant *streptococcal* protein G lacking the albumin-binding produced in *E. coli*
Number of IgG binding sites per ligand	3
MW of ligand	Approximately 22 kDa
pI of ligand	4.69
Degree of substitution	Approximately 2 mg protein G/ml
Static binding capacity	>20 mg sheep IgG/ml drained medium
Stability	37°C, 7 days
Matrix spherical	agarose, 4%
Average particle size	90 µm (45–165 µm)
Sterilization	Wash the packed column with 70% ethanol
Storage	20% ethanol at +4 to +8 °C

IV. Immunoglobulin Purification Procedure

Before use, prepare the following two solutions:

1. Binding Buffer A:
   Na_2HPO_4 20 mM
   NaCl 0.15 M
   pH 7.0
2. Elution Buffer B:
   Citric acid pH 2.0

This procedure is for a column of 0.5 ml bed volume. The volumes of reagents can be scaled up or down according to the size of the column.

1. Mix the slurry by gently inverting the bottle several times to suspend the resin completely.
2. Use a pipette to transfer appropriate volume of Protein G Resin slurry to a column. Allow the resin to settle and the storage buffer to drain from the column.
3. Add 5 ml of Binding Buffer A to equilibrate the Protein G Resin.
4. Dilute the sample with the same volume or more of Binding Buffer A before applying onto the Protein G column to maintain optimal ionic strength for binding.
5. Wash the column with 30 ml of Binding Buffer A.
6. Elute the antibody with 15 ml of Elution Buffer B. Immediately neutralize the eluted fractions with 1M Tris-HCl, pH 8.5 to pH 7.4.

Regeneration of the column.

1. Regenerate column by washing the column with 10 ml of Elution Buffer B followed by equilibration of the column with 5 ml of Binding Buffer A. Columns can be regenerated up to 10 times without significant loss of binding capacity.
2. For storage, wash column with 5 ml of PBS containing 0.02% sodium azide. Store column upright at 4°C.

V. Application Example

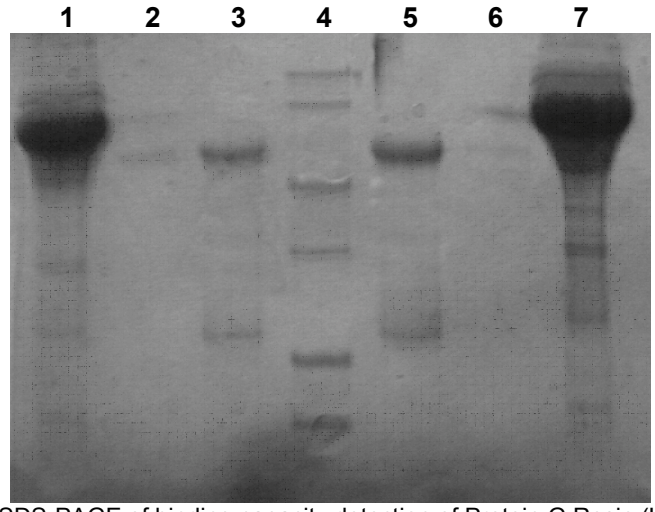

Fig 1. SDS-PAGE of binding capacity detection of Protein G Resin (L00209)
Lane 1. Flow (X corporation)
Lane 2. Wash (X corporation)
Lane 3. Elute (X corporation)
Lane 4. Molecular standard (KDa: 97, 66, 43, 31, 20, 14)
Lane 5. Elute (GenScript)
Lane 6. Wash (GenScript)
Lane 7. Flow (GenScript)

VI. Troubleshooting

Problem	Possible Cause	Solution
Flow of the column is exceedingly slow (i.e., <0.5 ml/minute)	Outgassing of buffers or sample on the column, which results in blockage of gel pores with microscopic air bubbles	Degas buffers and remove air bubbles from column (see Additional Information section for suggested Tech Tip protocol)
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest is at very low concentration	Use serum-free medium for cell supernatant samples
		Affinity purify the antibody using the specific antigen coupled to an affinity support.
Antibody of interest purified, but it is degraded (as determined by lack of function in downstream assay)	Antibody is sensitive to low-pH Elution Buffer	Increase pH of Elution Buffer.
	Downstream application is sensitive to neutralized Elution Buffer	Desalt or dialyze eluted sample into suitable buffer
No antibody detected in any elution fraction	Sample devoid of antibody species or subclass that binds to Protein G	Refer to the Binding Characteristics Table for Protein G (see Additional Information section)