Protein G Resin Cat. No. L00209

Technical Manual No. TM0209 **Version 07092010**

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1. Product Description

GenScript Protein G Resin is an affinity chromatography medium designed for easy, one-step purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. The recombinant protein G ligand is coupled to 4% highly cross-linked agarose. The static binding capacity of Protein G Resin is greater than 20 mg sheep IgG/ml settled resin. The dynamic binding capacity will vary depending on several factors such as target antibody, flow rate etc. Table 1 lists the characteristics of Protein G Resin.

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. The latter have been eliminated from recombinant protein G to reduce nonspecific binding. Although protein G has very similar tertiary structures to protein A, their amino acid compositions differ significantly, resulting in different binding characteristics. Protein G can 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.

Table 1. Characteristics of Protein G Resin

Resin Volume	5 ml settled resin (10 ml 50% slurry)
Ligand	Recombinant Streptococcal protein G lacking the albumin-binding sites expressed in *E. coli*
Number of IgG binding sites per ligand	3
M.W. of ligand	Approximately 22 kDa
PI of ligand	4.69
Degree of substitution	Approximately 2 mg protein G/ml settled resin
Static binding capacity	> 20 mg sheep IgG/ml settled resin
Matrix spherical	4% cross-linked agarose
Average particle size	90 µm (45-165 µm)
Storage solution	1X PBS containing 20% ethanol
Storage & Stability	12 months when stored unopened at 2-8 °C

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2. Operation

Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.45 µm filter before use.

Binding/Wash Buffer: 20 mM Na_2HPO_4, 0.15 M NaCl, pH 8.0

Elution Buffer: 0.1 M glycin, pH 2.5

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

3. Purification Procedure

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

Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascite fluid or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer.

Packing of Column

1) Resuspend completely the resin and transfer 1 ml slurry to a new column, in which 1 ml Binding/Wash Buffer was added in advance.
2) Allow the resin to settle down and the buffer to drain from the column.
3) Add 5 ml Binding/Wash Buffer onto the column to equilibrate the resin and drain the buffer with a flow speed of about 1 ml/min.

Column Purification

1) Apply the sample onto the column and drain the flow-through with a flow speed of about 1 ml/min. Collect the flow-through for measuring the binding efficiency to the resin, i.e. by SDS-PAGE.
2) Wash the column with 30 ml Binding/Wash Buffer and drain the buffer with a flow speed of about 2 ml/min**,** or until the absorbance of the effluent at 280 nm is stable.
3) Elute the immunoglobulins with 10-15 ml Elution Buffer and drain the eluate with a flow speed of about 1 ml/min. Collect the eluate and immediately neutralize to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

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Regeneration of Column

Regenerate the column by washing the resin with 10 ml Elution Buffer followed by equilibration with 5 ml Binding/Wash Buffer. Columns can be regenerated up to 10 times without significant loss of binding capacity.

Storage

Store regenerated Protein G Resin in Binding/Wash Buffer containing 20% ethanol at 2°C to 8°C. **Do not freeze**.

4. Troubleshooting

Problem	Possible Cause	Solution
The flow rate of the column is very low (<0.5 ml/minute).	Tiny air bubbles from buffer or particles from sample block the gel pores.	De-gas buffers and samples. Do not allow the column to dry.
A considerable amount of sample has been loaded, but no specific antibody of interest is detected.	The concentration of antibody of interest is very low.	Purify the antibody using the specific antigen coupled to a resin (i.e., High-Affinity Iodoacetyl Resin, Cat. No. L00403).
The antibody is degraded.	The antibody is sensitive to low-pH elution buffer	Neutralize the eluted fractions with Neutralization Buffer immediately after elution.
No antibody is detected in any elution fraction.	The IgG subclass does not bind to protein G.	Try other affinity chromatography media to purify the antibody, such as Protein A Resin or Protein L Resin.

5. Related Products

Cat. No.	Product Name
L00210	Protein A Resin
L00400	Ultra Protein A Resin
L00239	Protein L Resin
L00405	Chicken IgY Precipitating Resin
L00223	High Affinity Ni-Charged Resin
L00206	Glutathione Resin
L00353	Streptavidin Resin
L00272	IminoBiotin Resin
L00207	GST Fusion Protein Purification Kit
L00208	Protein Expression and Purification Kit
L00403	High-Affinity Iodoacetyl Resin
L00404	High-Affinity Antibody Purification Kit

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