Version：02
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Product Name: Protein A Resin
Cat. No.：L00210

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I. Product Description

GenScript Protein A 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. Protein A Resin can also be used for immunoprecipitation of proteins, protein complexes or antigens. The recombinant protein A ligand is coupled to 4% agarose. The coupling is optimized to give high binding capacity for immunoglobulins. The static binding capacity of Protein A Resin is greater than 20 mg human IgG/ml settled resin. The binding capacity will vary depending on several factors such as target antibody, flow rate etc. Table 1 lists the characteristics of Protein A Resin.

Protein A, a bacterial cell wall protein isolated from *Staphylococcus aureus*, binds to mammalian IgGs mainly through Fc regions. Native protein A has five IgG binding domains and many other domains with unknown functions. Recombinant protein A contains five high affinity IgG binding domains with other non-essential domains removed to reduce nonspecific binding. Since only the Fc region is involved in binding, the Fab region is available for binding antigens.

Table 1. Characteristics of Protein A Resin

Resin Volume	5ml settled resin (10 ml 50% slurry)
Ligand	Recombinant *Streptococcal* protein A expressed in *E. coli*
Number of IgG binding sites per ligand	5
M.W. of ligand	Approximately 34 kDa
PI of ligand	5.17
Degree of substitution	Approximately 2 mg protein A/ml settled resin
Static binding capacity	> 20 mg human IgG/ml settled resin
Matrix spherical	Agarose, 4%
Average particle size	90 μm (45-165 μm)
Storage solution	20% ethanol
Storage conditions	2-8 °C; **Do not freeze**

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II. Operation

2.1 Buffer Preparation

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

Binding/Wash Buffer:	0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.0
Elution Buffer:	0.1 M glycine, pH 3.0
Neutralization Buffer:	1 M Tris-HCl, pH 8.5

2.2 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.

2.2.1 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.

2.2.2 Packing of Column

(1) Completely resuspend 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 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 1ml/min.

2.2.3 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 antibody with 10-15 ml Elution Buffer and drain the eluate with a flow speed of about 1 ml/min. Collect the eluate containing the target immunoglobulin and immediately neutralize to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

2.2.4 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.

2.3 Storage

Store regenerated Protein A Resin in 20% ethanol at 2°C to 8°C. **Do not freeze**.

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III. Trouble Shooting

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 A.	Try other affinity chromatography media to purify the antibody, such as Protein G Resin or Protein L Resin

IV. Related Products

Cat. No.	Product Name
L00433	Monofinity A Resin
L00464	Protein A Resin FF
L00209	Protein G Resin
L00664	Protein G Resin FF
L00239	Protein L Resin
L00223	High Affinity Ni-Charged Resin
L00465	Ni Resin FF
L00666	High Affinity Ni-Charged Resin FF
L00353	Streptavidin Resin
L00206	Glutathione Resin
L00207	GST Fusion Protein Purification Kit
L00403	High-Affinity Iodoacetyl Resin

For research and manufacturing use. Direct human use, including taking orally and injection are forbidden.