Version：02

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Protein A Resin FF Prepacked Column
Cat. No. L00680

I Product Description

GenScript Protein A Resin FF is an affinity chromatography media designed for easy, one-step purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. Protein A Resin FF can also be used for immunoprecipitation of proteins, protein complexes or antigens. The recombinant protein A ligand is coupled to 4% highly cross-linked agarose. The coupling is optimized to have a high binding capacity for immunoglobulins. The static binding capacity of Protein A Resin FF is greater than 40 mg human 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 A Resin FF Prepacked Column.

Protein A, a bacterial cell wall protein isolated from *Staphylococcus aureus*, binds to mammalian IgGs mainly at their Fc region. 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 to recombinant protein A, the Fab region is available for binding antigens.

Table 1. Characteristics of Protein A Resin FF Prepacked Column

Resin Volume	1 ml x 2(L00680-12);5ml x 1(L00680-51);5ml x 5(L00680-55)
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 5 mg protein A / ml settled resin
Static binding capacity	> 40 mg human IgG/ ml settled resin
Matrix component	Agarose, 4% highly cross-linked
Average particle size	90 µm (45-165 µm)
Storage solution	20% ethanol
Storage conditions	2-8 °C

II Buffer Preparation

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Water and chemicals used for buffer preparation should be of the highest purity. It is recommended to filter the buffers by passing 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

III Purification Procedure

This procedure is optimized for a column of 1 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, ascites fluid or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer.

● Column Purification

1. Add the sample into the column and drain the flow-through with a flow speed of about 1 ml/min. Collect the flow-through to measure 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).

● 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.

IV Storage

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

V Trouble Shotting

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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 are blocking 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 the 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.

VI Ordering Infomation

Product Name	Cat. No.
Monofinity A Resin	L00433
Protein A Resin FF	L00464
Protein G Resin FF	L00664
Protein L Resin	L00239
Protein G Resin FF Prepacked Column	L00681
Protein A MagBeads MX	L00672
Protein G MagBeads MX	L00673

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

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