Protein A Resin

Technical Manual No. 0205 **Version 20060721**

I. Description

Protein A Resin (Cat. No. L00210) is useful for affinity purification and isolation of IgG. 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 of unknown functions. Recombinant Protein A contains mainly five high affinity (Ka = 10^8/M) IgG binding domains with other non-essential domains removed to reduce nonspecific binding. Additionally, a 3×Cys tag was engineered to the C-terminal of recombinant protein A to facilitate its immobilization or conjugation.

II. High lights

- Broad IgG binding spectrum.
- High IgG-binding capacity.
- Milder elution condition than protein G Resin.
- Resin is reusable (up to 10 times).

III. Characteristics of Protein A Resin

Ligand	Recombinant *Streptococcal* protein A produced in *E. coli*
Number of IgG binding sites per ligand	5
MW of ligand	Approximately 43 kDa
PI of ligand	5.17
Degree of substitution	Approximately 2 mg protein A/ml
Static binding capacity	>20 mg Porcine IgG/ml drained medium
Stability	2 - 8°C, 6 months
Matrix spherical	Agarose, 4% cross-linked
Average particle size	90 µm (45–165 µm)
Storage solution	1X PBS containing 20% ethanol
Storage conditions	2 - 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 0.1 M
pH 3.0

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.

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

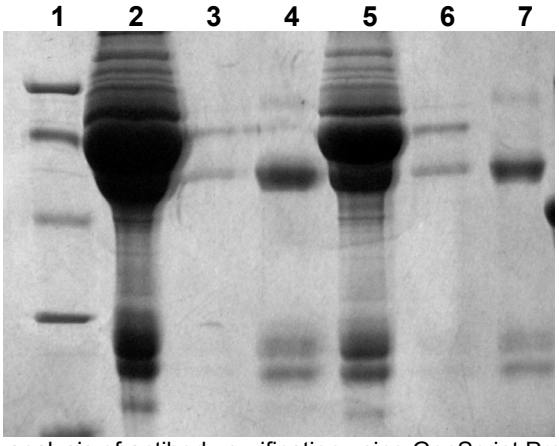

Fig.1. SDS-PAGE analysis of antibody purification using GenScript Protein A Resin (L00210)
Lane 1. Molecular standard (KD: 97 66 43 31 20).
Lane 2. Flow-through (GenScript)
Lane 3. Wash (GenScript)
Lane 4. Elute (GenScript)
Lane 5. Flow (X corporation)
Lane 6. Wash (X corporation)
Lane 7. Elute (X corporation)

VI. Troubleshooting

Problem	Possible Cause	Solution
Flow rate of the column is very low (i.e., <0.5 ml/minute).	Tiny air bubbles from buffers or sample block gel pores.	Degas buffers and samples. Do not allow the column to dry.
Considerable antibody purified, but no specific antibody of interest detected.	The concentration of antibody of interest is very low.	Affinity purify the antibody using the specific antigen coupled to an column.
Antibody is degraded.	Antibody is sensitive to low-pH Elution Buffer	Neutralize the elute immediately after elution.
No antibody detected in any elution	The IgG subclass does not bind to	Use other affinity column to purify

Fraction.	Protein A.	the antibody.

VII. Order information

Protein A Resin (10 ml of 50%slurry): L00210

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