Protein A-Agarose Resin

Ordering Information:	Storage Conditions:
Product Number: 55000 (1mL), 55005 (5mL)	2 to 8°C, DO NOT FREEZE.

Introduction:

Protein A has good affinity with most immunoglobulins in Fc region of the heavy chain. Protein A agarose resin has been used as a powerful tool to purify the immunoglobulins from biological fluids, especially for purification of immune complexes.

Specifications:

Ligand	Protein A
Particle size	45~160 μm
Binding Capacity	~20 mg human IgG/ml medium
pH Stability Working Range	3–9
Matrix	Cross-linked agarose, 4% beaded supplied as a 50% slurry (e.g., 1mL of settled resin is equivalent to 2mL of 50% slurry) containing 0.09% sodium azide
Storage Conditions	2 to 8 °C (DO NOT FREEZE), This product is stable for 1 year at 4°C.

Protocol:

- 1. <u>Equilibrium</u>: Open the bottom outlet of the column, drain the storage buffer under gravity, and then wash the resin with 10 column volumes (CV) of binding buffer.
- Binding: Seal the bottom of the column, dilute the sample by 2 folds with binding buffer, and add the sample to the column. Cap the top of the column, mix the sample with resin well and incubate at room temperature for 10~20 minutes.
- 3. <u>Washing:</u> Wash the column with 10CV of PBS until the absorbance (280nm) of the flow through is near or at background levels.
- <u>Elution and neutralization</u>: Elute the immunoglobulins in a stepwise manner. Seal the bottom of the column, add 500μL 0.1 M glycine buffer pH 3.0 per step, incubate for 10 minutes, and collect the eluate in 1.5ml tubes containing 50μL 1M Tris-HCl, pH 9.0 per ml fraction. Total wash with 5CV elution buffer.
- 5. <u>Regeneration</u>: Add another 5 CV 0.1 M glycine buffer pH 3.0 until the absorbance (280nm) of the flow through is near or at background levels, followed by washing the column with at least 10CV storage buffer. Store resin in storage buffer at 4°C.

Buffers Suggested:

- 1. Bindbing Buffer: PBS or 50mM Tris -HCl pH=7.5
- 2. Elution Buffer: 0.1 M glycine buffer pH 3.0
- 3. Neutralization Buffer: 1M Tris-HCl, pH 9.0
- 4. Washing Buffer: PBS
- 5. Storage Buffer: PBS+0.09% Sodium Azide