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Protein A Agarose Beads for Antibody Purification

Store at 2-8°C

Cat. No.	Description	Quantity
G243	Protein A Agarose Resin	5ml
G244	Protein A Agarose Resin	25ml
G245	Protein A Agarose Column	1 X 1ml

Product Description

Affinity chromatography is a method of separating biochemical mixtures based on highly specific biological interactions. The Protein A Agarose is developed for purification of classes, subclasses and fragments of immunoglobulins, especially IgGs of different species. Protein A is a cell wall component isolated from *Staphylococcus aureus* that contains five antibody-binding domains with affinity for IgG, some IgA and IgM. This high affinity domain-Fc region of the immunoglobulins interaction makes one-step purification possible for proteins under native conditions. The immunoglobulins bind to Protein A, which are immobilized onto a solid support. Impurities are removed and the purified immunoglobulins can be eluted by decreasing the pH.

Protein A Agarose Resins are supplied as a suspension in 20% ethanol and can be used in batch or column purifications. Protein A Agarose Columns are in ready-to-use format for purification of immunoglobulins by gravity flow.

Technical Specifications for Protein A Agarose Beads

Beads (Geometry, Size)	Spherical, Standard: ~40-180 um
Agarose %	4% Agarose
Ligand Density	~3mg Protein A /ml resin
Binding Capacity	~25 mg human IgG /ml resin
Column Material	Polypropylene and polyethylene frit
Storage Temperature	2 - 8 °C

Shipping and Storage

Upon arrival, the Protein A Agarose Resin/ Column should be stored at 2–8°C. Do not freeze. For long term storage after use, it is recommended to leave the Protein A Agarose Resin/ Column in 20% ethanol or other preservatives.

General Protocol

1. Elimination of the preservative: Wash the beads with 5 – 10 bead volumes/ column volumes of distilled water.
2. Equilibration of the resin: Equilibrate the beads with 5 – 10 bead volumes/ column volumes of the binding buffer.

   NOTE: The most frequently used buffers are sodium phosphate (25mM) or Tris (50mM) at neutral pH; however, the choice of buffer depends on the properties of the immunoglobulin of interest. Alternatively, alkaline buffers such as PBS (100mM) and NaCl (150mM) at pH 7.2 can be used to increase the interaction between Protein A and the immunoglobulin.
3. Application of the sample: Once the resin has been washed and equilibrated, the immunoglobulin sample can be applied to the beads/ column. In column purification, the recommended flow rate is 0.5 ml - 1.0ml /min.

   NOTE: Binding capacity is affected by parameters such as sample concentration, binding buffer, and the flow rate during sample application. Generally, increased contact time between the resins and the sample will facilitate binding.
4. Washing of resin: Wash the beads with 5 – 10 bead volumes/ column volumes of the binding buffer. This step will wash off any non-specific proteins bound to the beads and increase the purity of the final protein product.
5. Elution of the pure protein: Reducing the pH will elute the retained immunoglobulin from the beads/ column. Most immunoglobulins are eluted in glycine (100mM) or citric acid buffer (100mM) at pH 3.0.

Recommendations for Optimal Results

- Work at low temperatures such as 4°C to minimize degradation of immunoglobulins.
- Add Tris (1M) at pH 9.0 to the purified immunoglobulin will neutralize the eluted fractions and ensure protein stability.
- Dilute the sample 1:1 with the binding buffer before sample application will help maintain proper ionic strength and pH for optimal binding.
- Protein A Agarose Resins are fragile, therefore gentle handling is important to ensure the optimal result.