

Recombinant Protein A Agarose, Fast Flow

(10ml packed beads)

Catalog # 16-156

Lot # 16981

Product Description: Recombinant Protein A covalently coupled by alkylamine linkage to highly cross-linked 6% agarose beads. Suitable for medium and low-pressure chromatography. Stable in all aqueous buffers used in Protein A chromatography. Recommended for flow rates from 30 to 400cm/hr.

Storage and Stability: Stable for 1 year at 4°C from date of shipment.

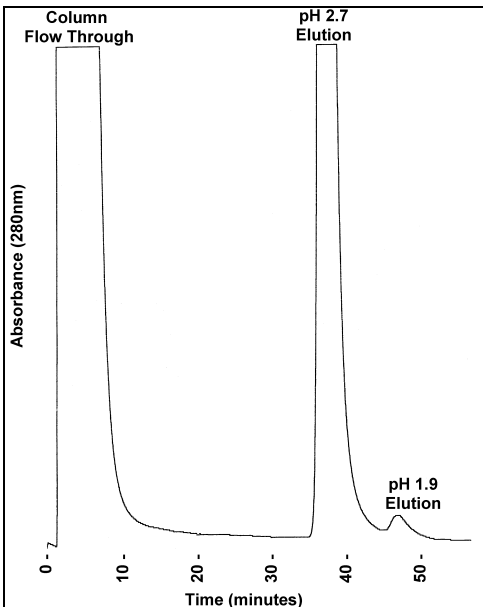
Physical Form: Liquid Suspension. Prior to use, wash the agarose beads with an appropriate buffer to remove the thimerosal.

Quantity and Formulation: 10ml packed beads, containing 3.5mg/ml recombinant Protein A. Suspended as a 50% slurry in phosphate-buffered saline containing 0.01% thimerosal. Binding capacity of 20mg human IgG/ml agarose.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

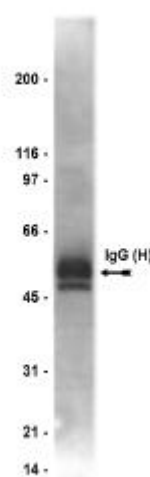
Quality Control Testing

Antibody Purification: A 1.7ml column was packed with recombinant protein A agarose, fast flow, and the IgG from a 1.5ml sample of rabbit serum was purified using a Waters™ 650E Advanced Protein Purification System.



Additional Research Applications

Immunoprecipitation: Use 100µl (50µl packed beads) to capture the immunoprecipitation immunocomplex.



Elution Profile and Analysis:

1.5ml of rabbit antiserum was purified using Recombinant Protein A, Fast Flow. The column eluent was monitored with an UV detector and recorded at 280nm. A 0.5µl sample of the pooled purified IgG was separated by SDS-Page on a 4-20% gradient gel and transferred to nitrocellulose. Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. The arrow indicates the IgG heavy chain. Similar results were seen with Coomassie Stain.

Immunoprecipitation Protocol

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly $1\mu\text{g}/\mu\text{l}$ total cell protein in a microcentrifuge tube with PBS.
2. Add antibody of choice to $500\mu\text{g}$ - 1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding $100\mu\text{l}$ of washed **recombinant protein A agarose** bead slurry ($50\mu\text{l}$ packed beads).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at $14,000 \times g$), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in $50\mu\text{l}$ 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.

Antibody Purification Protocol

1. 0.45μ filter (and degas all buffers prior to starting purification).
2. Pack **recombinant protein A agarose** into the column of choice, following the column manufacturer's instructions.
3. Wash and equilibrate packed protein A agarose with 10-20 column volumes of Tris buffered saline, pH 7.4 (TBS) at $1\text{ml}/\text{minute}$ flow rate.
4. Load sample containing IgG of interest onto column.
5. Wash column with 10-20 column volumes of TBS to remove unbound and non-specifically bound proteins. This can be determined by measuring the absorbance at 280nm with an inline UV detector. Alternatively, a post column sample can be collected and checked for protein.
6. Elute the bound IgG with 10ml of 50mM glycine pH 2.7, collecting 1ml fractions in tubes containing a antibody neutralization buffer (1M Tris, pH 8.0, 1.5M NaCl, 1mM EDTA). Eluted IgG can be determined by monitoring the absorbance at 280nm . **NOTE:** Some antibodies bind with high affinity to Protein A and will not elute at pH 2.7, in these cases, 50mM Glycine pH 1.9 may be used.
7. Wash the column with 10-20 volumes of TBS to restore the agarose column to neutral pH.
8. Store the column in TBS containing either 0.02% sodium azide or 0.01% thimerosal.