



cell signaling solutions

## Certificate of Analysis

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### Protein A Agarose, Fast Flow

(10ml packed beads)

Catalog # 16-156

Lot # 27578

**Product Description:** Recombinant Protein A covalently coupled to highly cross-linked 4% agarose beads. Suitable for medium and low-pressure chromatography. Stable in all aqueous buffers used in Protein A chromatography. Recommended for flow rates from 50 to 300cm/hr.

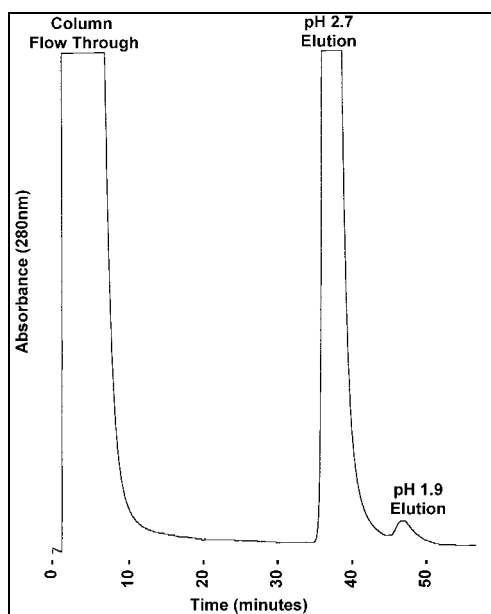
**Quantity and Formulation:** 10ml packed beads, containing 4.2mg/ml recombinant Protein A. Suspended as a 50% slurry in TBS, pH 7.4 containing 0.05% sodium azide for a final volume of 20ml. Binding capacity of 30mg human IgG/ml agarose. Liquid Suspension.

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

**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

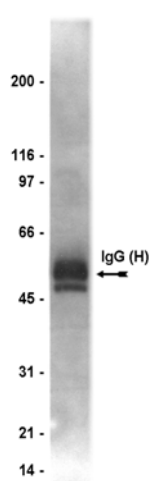
### Quality Control Testing

**Antibody Purification:** Representative elution profile from a previous lot using a 1.7ml column of recombinant protein A agarose, fast flow, and the IgG from a 1.5ml sample of rabbit serum. The IgG 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:

Representative gel from a previous lot. 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 $\mu$ g/ $\mu$ l total cell protein in a microcentrifuge tube with PBS.
2. Add antibody of choice to 500 $\mu$ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu$ l (50 $\mu$ l packed beads) of washed **Protein A Agarose, Fast Flow**, bead slurry.
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 x 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 60 $\mu$ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

### Antibody Purification Protocol

1. Use a 0.45 $\mu$  filter to filter all buffers.
2. Degas all buffers prior to starting purification.
3. Pack **Protein A Agarose, Fast Flow**, into the column of choice, following the column manufacturer's instructions.
4. Wash and equilibrate packed protein A agarose with 10-20 column volumes of Tris buffered saline, pH 7.4 (TBS) at 1ml/minute flow rate.
5. Load sample containing IgG of interest onto column.
6. 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.
7. Elute the bound IgG with 10ml of 50mM glycine pH 2.7, collecting 1ml fractions in tubes containing an 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.
8. Wash the column with 10-20 volumes of TBS to restore the agarose column to neutral pH.
9. Store the column in TBS containing either 0.02% sodium azide or 0.01% thimerosal.