

## Certificate of Analysis

### Protein G Agarose, Fast Flow

(10ml packed beads)

Catalog # 16-266

Lot # 0610042813

**Product Description:** Protein G covalently coupled by cyanogen bromide to highly cross-linked 4% agarose beads. Suitable for medium and low pressure chromatography. Stable in all aqueous buffers used in Protein G chromatography. Maximum linear flow rate  $\leq 1300$  cm/hr. Recommended for flow rates from 30 to 400cm/hr. Useful for purifying IgG from mouse, sheep and rabbit.

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

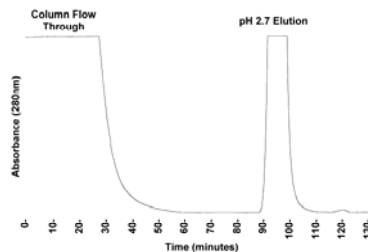
**Physical Form:** Liquid Suspension. Prior to use, wash the agarose beads with an appropriate buffer to remove the ethanol.

**Quantity and Formulation:** 10 mL packed beads, containing 2 mg/mL Protein G suspended as a 50% slurry in distilled water containing 20% ethanol for a final volume of 20 mL. Binding capacity of 20 mg human IgG/mL agarose.

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

### Quality Control Testing

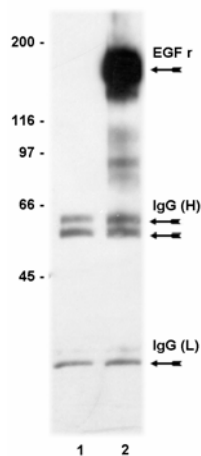
**Antibody Purification:** A mouse monoclonal IgG was purified from hybridoma supernatant fraction using a 12 mL column of Protein G Agarose, Fast Flow and a Waters™ 650E Advanced Protein Purification System.



#### Elution Profile and Analysis:

Mouse IgG was purified from 2L of hybridoma supernatant fraction using Protein G Agarose, Fast Flow. The column eluent was monitored with an UV detector and recorded at 280 nm.

**Immunoprecipitation:** 20  $\mu$ L of a 50% slurry (10  $\mu$ L packed beads) of a previous lot of Protein G Agarose, Fast Flow was used to capture a mouse IgG immuno-precipitation complex.



#### Immunoprecipitation:

Representative blot from a previous lot. Immunoprecipitation of Phosphotyrosine containing proteins contained in EGF-stimulated A431 cell lysate (Catalog # 12-110) using anti-phosphotyrosine, clone 4G10 (4  $\mu$ g, Catalog # 05-321) in conjunction with 20  $\mu$ L (50% slurry) Protein G Agarose, fast flow followed by immunoblot analysis using anti-phosphotyrosine, clone 4G10. Blot was developed using an HRP-conjugated secondary antibody and the ECL detection system.

### 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  $\mu\text{g}$ -1 mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding **20  $\mu\text{L}$  of washed Protein G Agarose** bead slurry (10  $\mu\text{L}$  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 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  $\mu\text{L}$  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. Filter using 0.45  $\mu\text{m}$  filter and degas all buffers prior to starting purification.
2. Pack **Protein G Agarose** into the column of choice.
3. Wash column with 10-20 column volumes of ice-cold TBS, pH 7.4 at 2.0 ml/minute.
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 280 nm with an inline UV detector. Alternatively, a post-column sample can be collected for protein. Continue washing with TBS until the absorbance is at background (< 0.2).
6. Elute the bound IgG with 50 mM glycine pH 2.7, collecting 1 mL fractions in tubes containing an antibody neutralization buffer (1 M Tris, pH 8.0, 1.5 M NaCl, 1 mM EDTA). Eluted IgG can be determined by monitoring the absorbance at 280 nm. **NOTE:** Some antibodies bind with high affinity to Protein G and will not elute at pH 2.7, in these cases, 50 mM Glycine pH 1.9 may be used.
7. Wash the column with 10-20 volumes of TBS to bring the agarose back to neutral pH.
8. Store the column in TBS containing either 0.02% sodium azide or 0.01% thimerosal.