



cell signaling solutions

Certificate of Analysis

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Protein G Agarose, Fast Flow

(10ml packed beads)

Catalog # 16-266

Lot # 24733

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.

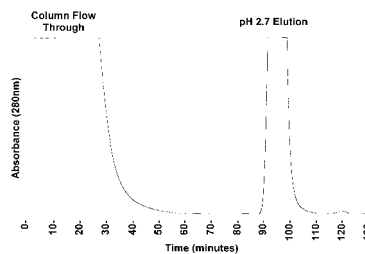
Quantity and Formulation: 10ml packed beads, containing 2mg/ml Protein G suspended as a 50% slurry in distilled water containing 20% ethanol for a final volume of 20ml. Binding capacity of 22mg human IgG/ml agarose.

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

Quality Control Testing

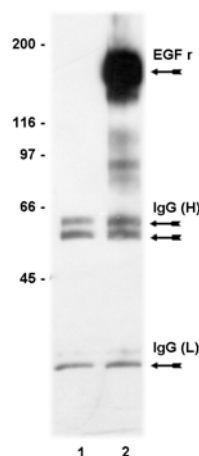
Antibody Purification: A mouse monoclonal IgG was purified from hybridoma supernatant fraction using a 12ml column of Protein G Agarose, Fast Flow and a Waters™ 650E Advanced Protein Purification System.

Immunoprecipitation: 20 μ l of a 50% slurry (10 μ l packed beads) of a previous lot of Protein G Agarose, Fast Flow was used to capture a mouse IgG immunoprecipitation complex.



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 280nm.



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 μ g, Catalog # 05-321) in conjunction with 20 μ 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 $500\mu\text{g}$ - 1mg 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 $14,000 \times g$), 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 $60\mu\text{l}$ 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 0.45μ 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 280nm 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 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 G 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 bring the agarose back to neutral pH.
8. Store the column in TBS containing either 0.02% sodium azide or 0.01% thimerosal.