



cell signaling solutions

Certificate of Analysis

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Protein A Agarose

(10ml packed beads)

Catalog # 16-125

Lot # 23906

Description and Formulation: 10ml packed beads containing sufficient covalently-linked Protein A for a binding capacity of 20 ± 2 mg human IgG/1ml settled agarose, as determined by an independent laboratory. Protein A is linked by a proprietary method to minimize leakage to less than 3ng/ml as determined by ELISA. Provided as a 50% gel slurry for a final volume of 20ml. Suspended water containing 0.01% thimerosal.

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

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

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

Quality Control Testing and Research Applications

Immunoprecipitation: Previous lots were tested by using 100 μ l of the gel slurry with monoclonal anti-Phosphotyrosine (Catalog # 05-321) to immunoprecipitate phosphotyrosine containing proteins from an EGF-stimulated A431 cell lysate (Catalog # 12-302).

Affinity Purification of IgG: A previous lot was tested by using 5ml of the gel slurry to quantitatively capture the IgG from 10ml of rabbit antiserum.

Immunoprecipitation Protocol

1. Prepare a cell lysate at a concentration of about 1 μ g/ μ l of protein and add 500 μ g-1mg to a microfuge tube.
2. Add an appropriate amount of primary antibody to the tube.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ l packed beads) of washed Protein A agarose 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.
7. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
8. Resuspend the agarose beads in 60 μ l 2X Laemmli sample buffer and boil 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, or the agarose beads can then be frozen for later use and reboiled for 5 minutes prior to SDS-PAGE.