

Certificate of Analysis

Protein A Agarose
(10ml packed beads)
Catalog # 16-125
Lot # DAM1487575

Description and Formulation: 10 mL packed beads containing sufficient covalently-linked Protein A for a binding capacity of 20 ± 2 mg human IgG/mL settled agarose, as determined by an independent laboratory. Protein A is linked by a proprietary method to minimize leakage to less than 3 ng/mL as determined by ELISA. Provided as a 50% gel slurry for a final volume of **20 mL**. Suspended in 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: This lot was tested using the PP2A Immunoprecipitation Phosphatase Assay Kit (Catalog # 17-313). Previous lots were tested by using 100 μ L of the gel slurry with monoclonal anti-Phosphotyrosine, clone 4G10TM (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 5 mL of the gel slurry to quantitatively capture the IgG from 10 mL 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-1 mg 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.

"Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals."