

**Recombinant Protein A-Agarose  
(10ml packed beads)**

**Catalog # 16-125**

**Lot # 16518**

**Background:** Recombinant Protein A covalently bound to agarose by alkylamine linkage.

**Quantity and Formulation:** 10ml packed beads containing approximately 3mg/ml recombinant Protein A. Provided as a 50% gel slurry for a final volume of 20ml. Suspended in phosphate-buffered saline (PBS), containing 0.1% thimerosal. Binding capacity of 18mg/ml human IgG.

**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 by using 100µl of the gel slurry with monoclonal anti-Phosphotyrosine (Catalog #05-321) to immunoprecipitate phosphotyrosine containing proteins from an EGF-stimulated A431 cell lysate.

**Affinity Purification of IgG:** This lot was tested by using 5ml of the gel slurry to quantitatively capture the IgG from 6ml of rabbit antiserum.

### **Immunoprecipitation Protocol**

1. Prepare a cell lysate at a concentration of about 1µg/µl of protein and add 500µg-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 **100µl of washed Protein A agarose bead slurry** (50µl 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.
7. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
8. Resuspend the agarose beads in 50µl 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.