For research purposes only, not for human use

Product Data Sheet

Protein A Agarose IP Reagent

Catalog #	Source	Reactivity	Applications
CRG1018		N/A	IP

Description Protein A is provided as an agarose conjugate for use in immunoprecipitation only. The product is provided as 0.5 ml agarose in 2.0 ml PBS. Protein A-Agarose is pre-blocked with BSA to reduce non-specific immunoglobulin binding. Sufficient product is provided for 100 immunoprecipitation reactions, to be used at 20 ul resuspended volume per reaction.

Specificity Protein A-Agarose is suitable for immunoprecipitation of mouse IgG2a, IgG2b and IgA, rabbit IgG, and human IgG1, IgG2 and IgG4.

Form Protein A-Agarose in PBS

Directions for Use

1. Incubate cultured cells (80 - 90% confluent monolayer in 100 mm cell culture plate, or approximately 2 - 5 x 10^7 suspension cells in flask) in methionine-free medium containing 5% dialyzed fetal calf serum for 1 hour at 37 °C. The same procedure can be used for cells labeled with other radioactive amino acids (e.g., 14C or 3H) or with γ32P-orthophosphate. Cell labeling must be carried out in medium lacking the relevant amino acid or in phosphate-free medium.
2. Remove medium and replace with 3 ml methionine-free medium containing 5% dialyzed fetal calf serum and 100 uCi/ml 35S-methionine. Incubate 1 hour at 37 °C. For some proteins a longer labeling period (up to 18 hours) is preferable.
3. Carefully remove radioactive medium with Pasteur pipette and wash cell monolayer with PBS.
4. Add 3 ml ice cold RIPA buffer to cell monolayer and incubate at 4 °C for 10 minutes. For suspension cells, add the RIPA buffer to washed cell pellet in a 15 ml conical centrifuge tube.
5. Disrupt cells by repeated aspiration through a 21 gauge needle and transfer to a 15 ml conical centrifuge tube.
6. Wash cell culture plate with additional 1.0 ml ice cold RIPA buffer and combine

with original extract.

7. Pellet cellular debris by centrifugation at 10,000xg for 10 minutes at 4 °C. Transfer supernatant to a fresh 15 ml conical centrifuge tube on ice. Preclear lysate (optional step) by adding 1.0 ug of the appropriate control IgG (normal mouse, rat, rabbit or goat IgG, corresponding to the host species of the primary antibody), together with 20 ul of resuspended volume of Protein A-Agarose. Incubate at 4 °C for 30 minutes.

8. Pellet beads by centrifugation at 2,500 rpm (approximately 1,000xg) for 5 minutes at 4 °C. Transfer supernatant (cell lysate) to a fresh 15 ml conical centrifuge tube on ice.

9. Transfer 1 ml of the above cell lysate, or approximately 100 - 500 ug total cellular protein, to a 1.5 ml microcentrifuge tube. Add 1 - 10 ul (i.e., 0.2 - 2 ug) primary antibody (optimal antibody concentration should be determined by titration) and incubate for 1 hour at 4 °C.

10. Add 20 ul of resuspended volume of Protein A-Agarose. Cap tubes and incubate at 4° C on a rocker platform or rotating device for 1 hour to overnight.

11. Collect immunoprecipitates by centrifugation at 2,500 rpm (approximately 1,000xg) for 5 minutes at 4 °C. Carefully aspirate and discard radioactive supernatant.

12. Wash pellet 4 times with 1.0 ml RIPA buffer (more stringent) or PBS (less stringent), each time repeating centrifugation step above.

13. After final wash, aspirate and discard supernatant and resuspend pellet in 40 ul of 1x electrophoresis sample buffer.

14. Boil samples for 2 - 3 minutes and analyze 20 ul aliquots by SDS-PAGE and autoradiography. Unused samples may be stored at -20 °C.

15. Optional: After boiling, samples may be centrifuged to pellet the agarose beads followed by SDS-PAGE analysis of the supernatant.

Storage/Stability Store at 4°C for one year, do not freeze.