#A10001

Protein A/G-Agarose

☐ 1ml (25-33 immunoprecipitation)

☐ 5ml (125–165 immunoprecipitation)

☐ 25ml (625–825 immunoprecipitation)

Orders ■ 400-6123-828
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PRODUCT INFORMATION

The Protein A/G-Agarose is provided as an agarose conjugate for use in immunoprecipitation or purification. The product is provided as 0.5 ml agarose in 1 ml PBS buffer with 0.05% Sodium Azide. Protein A/G-Agarose is pre-blocked with BSA to reduce non-specific immunoglobulin binding. Sufficient product is provided for 25-33 immunoprecipitation reactions, to be used at 30-40 µl resuspended volume per reaction.

Protein A/G is a genetically engineered protein (MW ~ 50,500; apparent MW by SDS-PAGE ~ 40,000-45,000) that combines the IgG binding profiles of both Protein A and Protein G. The secreted Protein A/G contains four Fc-binding domains from Protein A and two from Protein G. In addition, Protein A/G binding to immunoglobulins is not as pH dependent as Protein A.

SPECIFICITY

Protein A/G-Agarose is suitable for immunoprecipitation of mouse IgG1, IgG2a, IgG2b and IgG3, rat IgG1, IgG2a, IgG2b and IgG2c, rabbit and goat polyclonal Abs, and human IgG1, IgG2, IgG3 and IgG4.

STORAGE

Store the product at 4°C. Stable for one year from the date of shipment.

IMMUNOPRECIPITATION PROCEDURE

1. Wash adherent cells twice in the dish or flask with ice-cold PBS and drain off PBS. Wash non-adherent cells in PBS and centrifuge at 800 to 1000 rpm in a table-top centrifuge for 5 minutes to pellet the cells.
2. Add ice-cold modified RIPA buffer to cells (1 mL per 10^7 cells/100 mm dish/150 cm^2 flask; 0.5 mL per 5 x 10^6 cells/60 mm dish/ 75 cm^2 flask).
3. Scrape adherent cells off the dish or flask with a plastic cell scraper. Transfer the cell suspension into a centrifuge tube, and pass 10~20 times through a 21 gauge needle.
4. Centrifuge the lysate at 14,000 x g in a pre-cooled centrifuge for 15 minutes. Immediately transfer the supernatant to a fresh centrifuge tube and discard the pellet.
5. To prepare Protein A/G-Agarose, wash the beads twice with PBS and restore to a 50% slurry with PBS. It is recommended to cut the tip off of the pipette when manipulating agarose beads to avoid disruption of the beads.
6. Pre-clear the cell lysate by adding 20 µl of Protein A/G-Agarose slurry (50%) per 1 mL of cell lysate and incubating at 4 °C for 10 minutes on a rotator. Pre-clearing the lysate will reduce non-specific binding of proteins to the agarose when it is used later on in the assay.
7. Remove the Protein A/G-Agarose by centrifugation at 14,000 x g at 4°C for 5 minutes. Transfer the supernatant to a fresh centrifuge tube.
8. Determine the protein concentration of the cell lysate (e.g. if performing a Bradford assay one must dilute the cell lysate at least 1:10 before determining the protein concentration because of the interference of the detergents in the lysis buffer with the Coomassie-based reagent).
9. Dilute the cell lysate to approximately 1 µg/µl total cell protein with PBS to reduce the concentration of the detergents in the buffer. A more concentrated cell lysate (i.e., 10 µg/µl) may be necessary to immunoprecipitate a protein, which is found in low levels in a cell model.
10. Add the recommended volume of the immunoprecipitating antibody (see antibody datasheet for detailed information) to 500 µl (i.e., 500 µg) of cell lysate.
11. Gently rotate the cell lysate/antibody mixture for either 2 hours or overnight at 4°C on a rotator.
12. Capture the immunocomplex by adding 30-40 µl Protein A/G-Agarose slurry (15-20 µl packed beads) and gently rotating on a rotator for 1 or 2 hour at 4°C.
13. Collect the agarose beads by centrifugating for 3 minutes at 1000 rpm. Discard the supernatant and wash the beads 3 times with 800 µl ice-cold RIPA buffer (more stringent) or PBS (less stringent).
14. Resuspend the agarose beads in 30-60 µl 1x SDS loading buffer and mix gently.
15. The agarose beads are boiled for 10 minutes at 100°C to dissociate the immunocomplexes from the beads. The beads are collected by centrifugation and SDS-PAGE is performed with the supernatant. Unused samples may be stored at -20°C for later use.