ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

Recombinant Protein A+G-Agarose, affinity matrix suspension for IP

□ **Cat #.** PRAG25-AS-2 **Size**: 2 ml buffered suspension containing 0.5 ml beads/matrix (pre-blocked with BSA)

□ **Cat #.** PRAG25-AS-5 **Size**: 5 ml buffered suspension containing 1.25 ml beads/matrix (pre-blocked with BSA)

Protein A is a cell wall protein deriving from Staphylococcus aureus which exhibits unique binding properties for IgG from a variety of mammalian species and for some IgM and IgA as well. It binds with the Fc region of immunoglobulins through interaction with the heavy chain. It couples to a wide variety of reporter molecules including fluorescent dyes, enzyme markers, biotin, colloidal gold and radioactive iodine without affecting the antibody binding site. Recombinant Protein A was developed to increase the specificity of the molecule for IgG and is widely used both in research and bioprocessing.

Protein G is a bacterial cell wall protein isolated from group G *Streptococci*. Native Protein G contains two IgG-binding domains and sites for albumin and cell surface binding. The albumin and cell surface binding domains have been eliminated from purified Recombinant Protein G to reduce nonspecific binding and, therefore, can be used to separate IgG from crude samples. Optimal binding occurs at pH 5, although binding is also good at pH 7.0-7.2. Protein G has greater affinity than Protein A for most mammalian IgGs, it may be used for the purification of mammalian IgGs that do not bind well to Protein A. Protein G does not bind to human IgM, IgD and IgA.

Recombinant purified Protein-A and Protein G were coupled to Agarose using a unique immobilization chemistry that retains the maximum IgG-binding capacity and minimum Protein-A leaching (<5 ng Protein/ml).

Binding capacity-
~10-15 mg human IgG/ml of Protein A/G-Agarose.

Form and Storage

Protein-A-Agarose is supplied in PBS, pH 7.4 containing 0.02% azide as preservative (1:3; beads:buffer v/v suspension). It has been pre-blocked with bovine serum albumin (BSA) to minimize non-specific adsorption of antibodies. This product is specially formulated for immunoprecipitation (IP). Each vial of 2 ml is sufficient for 100 IP @20 ul suspension/IP. This volume should be as needed adjusted (20-50 ul suspension) for a given IP experiment. use wide bore pipette tip or a tip that is cut at the bottom to increase the opening.

Store at 4oC. Do not freeze and thaw. Stable for 1 yr from the date of shipment.

Specificity of Protein A/G

Protein A/G-agarose is suitable for IP of mouse (IgG1, IgG2a, IgG2b, IgG3, and IgA), rat (IgG1, IgG2a, IgG2b, IgG2c) rabbit and goat polyclonal antibodies and human (IgG1-4).

General Procedure of IP using cultured cells

1. Incubate cultured cells (from confluent monolayer or in suspension ~2–5 x 10^7 cells) and remove medium and wash with cold PBS two times.
2. Add 2-3 ml ice cold RIPA buffer to cell monolayer or washed cell pellet and incubate at 4° C for 10 minutes.
3. Disrupt cells by repeated aspiration through a pipette first and thenn 21 gauge needle and transfer to a 15 ml conical centrifuge tube.
4. Centrifuge the homogenous cell suspension in RIPA-buffer at 10,000xg for 10 minutes at 4° C. Transfer clear supernatant to a fresh 15 ml.
5. Pre-clear lysate (optional) by adding 1-5 µg of the appropriate control IgG (normal mouse, rat, rabbit or goat IgG, corresponding to the host species of the primary antibody), together with 20 µl of resuspended volume of Protein A/G PLUS-Agarose. Incubate at 4° C for 30 minutes.
6. Pellet beads by centrifugation at 2,500 rpm (approximately 1,000xg) for 5 minutes at 4° C. Transfer supernatant (cell lysate) to a fresh tube and keep it on ice or at 4oC.
7. Transfer 1 ml of the above cell lysate, or approximately 100–500 µg total cellular protein, to a 1.5 ml tube. Add 1–10 µl of the primary antibody (0.5-5 ug; optimal antibody concentration should be determined by titration) and incubate for 1 hour at 4° C.
8. Add 20 µl of resuspended volume of Protein A/G – Agarose (make sure to mix the suspension for 5-10 seconds before use). • • Close the tubes and incubate at 4° C on a rocker platform or rotating device for 1 hour to overnight.
9. Collect immunoprecipitates by centrifugation at 2,500 rpm (~ 1,000xg) for 5 minutes at 4° C. Carefully aspirate and discard supernatant.
10. Wash pellet 4 times with 1.0 ml RIPA buffer (more stringent) or PBS (less stringent), each time repeating centrifugation step above.
11. After final wash, aspirate and discard supernatant and resuspend pellet in 40 µl of 1x SDS-PAGE sample buffer. (reduced) and Boil samples for 2–3 minutes and analyze 20 µl aliquots by SDS-PAGE and autoradiography. Unused samples may be stored at -20° C. Note: After boiling, samples may be centrifuged to pellet the agarose beads to make it easier to use the supernatant for SDS-PAGE analysis.

Specificity of various IP reagents

Items	Cat#	Specificity
Protein A-Agarose	#PRTA16-AS-2	mouse IgG2a, IgG2b and IgA, rabbit polyclonal Abs, human IgG1, IgG2 and IgG4
Protein G-Agarose	#PRTG116-AS-2	mouse IgG1, IgG2a, IgG2b and IgG3, rat IgG1, IgG2a, IgG2b and IgG2c, rabbit and goat polyclonal Abs, human IgG1, IgG2, IgG3 and IgG4
Protein A+G Agarose	#PRTAG25-AS-2	As in Protein A and G
Protein L-Agarose		mouse, rat, human IgG, scFv and Fab, fragments, mouse and human IgM, IgE and IgA

For in vitro research use only

Related Material available for ADI

Antibodies to Protein A, G and L and HRP, biotin conjugates
Recombinant Protein A, G, L and coated plates
Protein A and G ELISA kits
PRAG25-AS-2-5 80429A

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