ALPHA DIAGNOSTIC INTERNATIONAL

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Product Data Sheet

Recombinant Protein L-Agarose, affinity matrix suspension for IP

Cat #. PRTL36-AS-2 **Size**: 2 ml buffered suspension containing 0.5 ml beads/matrix

Protein L (~36 Kda) is an immunoglobulin-binding protein isolated from the bacteria *Peptostreptococcus magnus*. Unlike Protein A and Protein G, which bind primarily through Fc regions (i.e., heavy chain) of immunoglobilins, Protein L binds Ig's through interactions with the light chains. Because no part of the heavy chain is involved in the binding interaction, Protein L binds a wider range of Ig classes than Protein A or G. Protein L binds to representatives of all classes of Ig's, including IgG, IgM, IgA, IgE and IgD. Single chain variable fragments (ScFv) and Fab fragments also bind to Protein L. Despite this wide-ranging binding capability with respect to Ig classes, Protein L is not a universal immunoglobilin-binding protein. Binding of Protein L to immunoglobulins is restricted to those containing kappa light chains (i.e., k chain of the VL domain). In humans and mice, kappa (k) light chains predominate. The remaining immunoglobulins have lambda (l) light chains. Furthermore, Protein L is effective in binding only certain subtypes of kappa light chains. For example, it binds human VkI, VkIII and VkIV subtypes but does not bind the VkII subtype. Binding of mouse immunoglobulins is restricted to those having VkI light chains.

The main application for immobilized Protein L is purification of monoclonal antibodies from ascites or culture supernatant that are known to have the kappa light chain. Protein L is extremely useful for purification of VLk-containing monoclonal antibodies from culture supernatant because it does not bind bovine, goat or sheep immunoglobilins, which are present in the media serum supplement. Protein L does not interfere with the antigen-binding site of the antibody, making it useful for immunoprecipitation assays, even using IgM. Protein-L can be utilized where Protein-A or G show poor binding.

Specificity of Protein L

Protein L only binds to Ig's containing light chains of type kappa I, III, and IV in human and kappa I in mouse. Protein L also may be specific for certain kappa subgroups in other species. Protein L binds scfv without interfering with antigen binding. Protein L binds weakly to rabbit Ig's and does not bind Ig's from bovine, goat or sheep; nor does it bind to lambda light chains.

Binding capacity-
~5-10 mg human IgG/ml settled volume of Protein -agarose.

Form and Storage

Protein-L-Agarose is supplied in PBS, pH 7.4 containing 0.02% azide as preservative (1:3; beads:buffer v/v suspension). 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 adjusted 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.

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 L-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 L –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	#PRTL36-AS-2	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

Protein A and G ELISA kits, Protein A/G-Agarose
Recombinant Proteins A, G, and L; Antibodies and conjugates

PRTL36-AS-2 90330A

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