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Protein L-Agarose Immunoprecipitation Reagent: sc-2336

PRODUCT

Protein L 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 buffer with 0.02% azide. Protein L-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 μ l resuspended volume per reaction.

REFERENCE

1. Kastern, W., et al. 1992. Structure of peptostrepto-coccal protein I and identification of a repeated immunoglobulin light chain-binding domain. *J. Biol. Chem.* 267: 12820-12825.

SPECIFICITY

Protein L-Agarose is suitable for immunoprecipitation of mouse, rat and human IgG, mouse, rat and human IgM, IgE and IgA proteins and scFv and Fab fragments.

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment.

PROCEDURE

- Incubate cultured cells (80–90% confluent monolayer in 100 mm cell culture plate, or approximately 2–5 x 10⁷ 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 γ ³²P-orthophosphate. Cell labeling must be carried out in medium lacking the relevant amino acid or in phosphate-free medium.
- Remove medium and replace with 3 ml methionine-free medium containing 5% dialyzed fetal calf serum and 100 μ Ci/ml ³⁵S-methionine. Incubate 1 hour at 37° C. For some proteins a longer labeling period (up to 18 hours) is preferable.
- Carefully remove radioactive medium with Pasteur pipette and wash cell monolayer with PBS.
- 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.
- Disrupt cells by repeated aspiration through a 21 gauge needle and transfer to a 15 ml conical centrifuge tube.
- Wash cell culture plate with additional 1.0 ml ice cold RIPA buffer and combine with original extract.
- 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. Pre-clear lysate (optional step) by adding 1.0 μ g of the appropriate control IgG (normal mouse, rat, rabbit or goat IgG, corresponding to the host species of the primary antibody), together with 20 μ l of resuspended volume of Protein L-Agarose. Incubate at 4° C for 30 minutes.

- 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.
- Transfer 1 ml of the above cell lysate, or approximately 100–500 μ g total cellular protein, to a 1.5 ml microcentrifuge tube. Add 1–10 μ l (i.e., 0.2–2 μ g) primary antibody (optimal antibody concentration should be determined by titration) and incubate for 1 hour at 4° C.
- Add 20 μ l of resuspended volume of Protein L-Agarose. Cap tubes and incubate at 4° C on a rocker platform or rotating device for 1 hour to overnight.
- Collect immunoprecipitates by centrifugation at 2,500 rpm (approximately 1,000xg) for 5 minutes at 4° C. Carefully aspirate and discard radioactive supernatant.
- Wash pellet 4 times with 1.0 ml RIPA buffer (more stringent) or PBS (less stringent), each time repeating centrifugation step above.
- After final wash, aspirate and discard supernatant and resuspend pellet in 40 μ l of 1x electrophoresis sample buffer.
- Boil samples for 2–3 minutes and analyze 20 μ l aliquots by SDS-PAGE and autoradiography. Unused samples may be stored at -20° C.
- Optional: After boiling, samples may be centrifuged to pellet the agarose beads followed by SDS-PAGE analysis of the supernatant.

SELECT PRODUCT CITATIONS

1. Liang, C.P., et al. 2004. Increased CD36 protein as a response to defective insulin signaling in macrophages. *J. Clin. Invest.* 113: 764-773.
2. Kameoka, S., et al. 2004. p54(nrb) associates with the 5' splice site within large transcription/splicing complexes. *EMBO J.* 23: 1782-1791.
3. Marg, A., et al. 2008. Microinjected antibodies interfere with protein nucleocytoplasmic shuttling by distinct molecular mechanisms. *Cytometry A.* 73A: 1128-1140.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

IMMUNOPRECIPITATION REAGENTS

PRODUCT	SPECIFICITY	CAT. #	AMOUNT
Protein A-Agarose	mouse IgG _{2a} , IgG _{2b} and IgA rabbit polyclonal Abs human IgG ₁ , IgG ₂ and IgG ₄	sc-2001	2.0 ml
Protein G PLUS-Agarose	mouse IgG ₁ , IgG _{2a} , IgG _{2b} and IgG ₃ rat IgG ₁ , IgG _{2a} , IgG _{2b} and IgG _{2c} rabbit and goat polyclonal Abs human IgG ₁ , IgG ₂ , IgG ₃ and IgG ₄	sc-2002	2.0 ml
Protein A/G PLUS-Agarose	all of the above Abs	sc-2003	2.0 ml
Protein L-Agarose	mouse, rat, human IgG, scFv and Fab fragments, mouse and human IgM, IgE and IgA	sc-2336	2.0 ml

Immunoprecipitation agarose conjugates are pre-blocked with BSA to reduce non-specific immunoglobulin binding and are provided at a concentration (0.5 ml agarose/2.0 ml) suitable for use at 20 μ l per immunoprecipitation reaction. Number of reactions: 100.