

## PRODUCT DATA SHEET



# Bioepitope<sup>R</sup> protein A agarose IP Reagent

**CATALOG NUMBER:** BD0046

**QUANTITY:** 2ml

**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  $\mu$ l 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.

**FORMAT:** PBS, 0.5ML agarose

**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 10<sup>7</sup> 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., <sup>14</sup>C or <sup>3</sup>H) or with <sup>32</sup>P-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  $\mu$ Ci/ml <sup>35</sup>S-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. Preclear lysate (optional step) by adding 1.0  $\mu$ 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  $\mu$ l of resuspended volume of Protein A-Agarose. Incubate at 4° C for 30 minutes.

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- 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 µg total cellular protein, to a 1.5 ml microcentrifuge tube. Add 1–10 µl (i.e., 0.2–2 µg) primary antibody (optimal antibody concentration should be determined by titration) and incubate for 1 hour at 4° C.
- Add 20 µl 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.
- 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 µl of 1x electrophoresis sample buffer.
- Boil samples for 2–3 minutes and analyze 20 µl 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.

**RESEARCH USE:** For research use only, not for use in diagnostic procedures.

### IMMUNOPRECIPITATION REAGENTS:

PRODUCT	SPECIFICITY	CAT. #	AMOUNT
protein A agarose	mouse IgG2a, IgG2b and IgA rabbit polyclonal Abs human IgG1, IgG2 and IgG4	BD0046	2ML
Protein G Agarose	mouse IgG1, IgG2a, IgG2b and IgG3 rat IgG1, IgG2a, IgG2b and IgG2c rabbit and goat polyclonal Abs human IgG1, IgG2, IgG3 and IgG4	BD0047	2ML
Protein A+G Agarose	all of the above Abs	BD0048	2ML

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