Immunoprecipitation Kit
Protein A-agarose

Catalogue #: IPK001A
Storage: 4—8 ºC
Size: Kit

Description:

The Protein A-agarose Purification Kit is designed for rapid purification of proteins expressed in cultured cells including mammalian cells, insect cells, yeast and E. coli. The easy-to-follow procedure is based on novel protein purification chemistry. Purification may take place under native conditions or under denaturing conditions depending on the solubility and/or desired application of the expressed protein. The purified protein can be used directly for enzymatic assays, protein biochemical analyses, SDS-PAGE, as well as other protein based applications.

Size: 30 standard assays

Kit components:

Components	*Name*	*Cat#*	*Size*
Component A	Protein A-agarose	IR003	1 mL
Component B	Binding Buffer	N/A	50 mL
Component C	Washing Buffer (5X)	N/A	50 mL
Component D	Elution Buffer	N/A	10 mL
Component E	Neutralization Buffer	N/A	1 mL

Reagents needed, but not provided in the kit:

- ✧ DTT (Cat. #: MC010)
- ✧ Phosphate Buffered Saline (PBS) (Cat. #: CC008)
- ✧ Proteinase Inhibitor Cocktails (Cat. #: MP027)

Precedures:

A. Preparation of Cell Lysates (for adherent mammalian cells)

1. Remove the growth medium from the cells to be analyzed. Rinse the cells twice with PBS buffer (Cat. #: CC008).
2. Add 10ml (10-cm plate), scrape the cells off the plate and transfer cells into 15-cm Folcon tube.
3. Centrifuge the sample at 1000 x g for 5 mins.
4. Discard the PBS, add lysis buffer (Cat. #: MP011T) supplemented with 1mM DTT (Cat. #: MC010) and Proteinase Inhibitor Cocktails (Cat. #: MP027) (10^6-10^7 cells/mL).
5. Incubate the cells for 15-30 minutes on a shaker.
6. Centrifuge the cell lysate for 10 minutes at 12,000 x g.
7. Transfer the supernatant to a 1.5ml eppendorf tube.

M&C Gene Technology (Beijing) Ltd., Phone: (010)8205-7786; (010)8693-7385
Fax: (010)8205-9875; E-mail: order@macgene.com; Online Support: support@macgene.com; URL: http://www.macgene.com

8. For immediate use, keep on ice. If the supernatant is not to be used immediately, store it at –70 °C.

B. Preparation of Protein A-agarose/antibody Complex and Immunoprecipitation

1. Thoroughly suspend the protein A-agarose beads.
2. Transfer 30ul of the gel suspension to a 1.5ml eppendorf tube. (For beads transfer, use plastic pipette tip with the end cut for about 2mm to allow the beads to be transferred).
3. Centrifuge the beads briefly to bring the beads to the bottom of the tube.
4. Wash the beads twice with 0.5 ml 1X Washing Buffer.
5. Add 0.5 ml Binding Buffer and up to 2 ug antibody against the protein of interest.
6. Incubate for 30 minutes with gentle rotating at RT.
7. Centrifuge the beads briefly to bring the beads to the bottom of the tube.
8. Discharge the supernatant and wash the beads twice with 0.5 ml 1X Washing Buffer.
9. Apply 0.5-1 ml of cell lysates (up to 1mg) to the beads. The lysates could be diluted with Binding Buffer.
10. Incubate for 2 hours-overnight with gentle rotating at 4ºC.
11. Centrifuge the beads briefly to bring the beads to the bottom of the tube.
12. Discharge the supernatant and wash the beads >5 times with 0.5 ml 1X Washing Buffer each.

C. Elution

Elution with 0.1 M glycine HCl, pH 2.5

1. Add up to 300ul Elution Buffer supplemented with 1 mM DTT to each sample.
2. Incubate the samples and controls with gentle shaking for 10 minutes at room temperature..
3. Centrifuge the beads for 30 seconds at 5,000 x g. Transfer the supernatants to a new tube containing Neutralization Buffer (1/10 volume of Elution Buffer).

Note: The procedure should be performed at room temperature. Do not leave the beads in this buffer >20 minutes.

Elution with SDS-PAGE Sample Loading Buffer

1. Add 30ul of 2X sample loading buffer (Cat. #: MP006.1) to each sample.
2. Boil the samples for 5 minutes.
3. Briefly votex the tube and centrifuge the samples at 5,000 x g for 30 seconds to pellet agarose.
4. Transfer the supernatants to a new tube.
5. The samples are ready for loading on SDS-PAGE and immunoblotting using Anti-FLAG or specific antibodies against the fusion protein or associated proteins.

Note: The procedure should be preformed at room temperature. Sample buffer should be at room temperature before use.

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