

FLAG-TAGGED PROTEIN PURIFICATION KIT

Catalogue Number: IPK002

Description: Anti-FLAG affinity gel is a purified mouse IgG₁ monoclonal antibody covalently conjugated to agarose by hydrazide linkage. The kit is designed for purification or immunoprecipitation of FLAG-tagged proteins from a variety of sources including mammalian cells, insect cells, bacteria and plant.

Size: 30 standard assays

Kit components:

Components	Name	Cat#	Size
Component A	Anti-FLAG Agarose Beads	IT001C	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)

Storage: 4–8°C

Experimental Procedures:

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 Falcon 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) (10⁶-10⁷ 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.
8. For immediate use, keep on ice. If the supernatant is not to be used immediately, store it at -70 °C.

B. Immunoprecipitation of FLAG-tagged Proteins

9. Thoroughly suspend the Anti-FLAG affinity agarose beads.

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10. 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).
11. Centrifuge the beads briefly to bring the beads to the bottom of the tube.
12. Wash the beads twice with 0.5 ml 1X Washing Buffer.
13. Add 500-1000ul of cell lysates (up to 1mg) to the beads. The lysates could be diluted with Binding Buffer.
14. Incubate the beads on shaker for 2 hours-overnight at 4°C.
15. Wash the beads five times with 1 mL Washing Buffer each. Centrifuge briefly to bring down the beads after each wash.
16. After the last wash, carefully remove the Washing Buffer.

C. Elution

Elution with 0.1 M glycine HCl, pH 2.5 (provided)

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 (not provided)

1. Add 30ul of 2X sample loading buffer (Cat# MP006.1) to each sample.
2. Boil the samples for 5 minutes.
3. Briefly vortex 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 performed at room temperature. Sample buffer should be at room temperature before use.

Elution with 3X FLAG peptide (not provided)

1. Prepare 3X FLAG elution solution. Dilute 3X FLAG peptide (Cat. #: PP011) in TBS buffer (Cat. #: MP011) to 150 ng/mL.
2. Add 100 uL of 3X FLAG elution solution to each sample and control resin.
3. Incubate the samples and controls with gentle shaking for 30 minutes at room temperature. Re-suspend the resin by gentle pipetting.
4. Centrifuge the resin for 30 seconds at 1,000 X g. Transfer the supernatants to fresh test tubes. Be careful not to transfer any resin.

For immediate use, store the supernatants at 2-8 °C. Store at -20 °C for long term storage.

