GST Fusion Protein Purification Kit

Protein Purification

Catalogure Number: IPK003

Description: Designed for purification of GST or GST-fusion proteins from a variety of sources including mammalian cells, insect cells, bacteria and plant.

Pack Size: 30 standard assays

Storage and Stability: Store at 4-8ºC for two years.

Kit components:

Components	*Name*	*Cat#*	*Size*
Component A	Glutathione Sepharose 4B	IR006	1mL
Component B	Binding Buffer		50mL
Component C	Washing Buffer (5X)		50mL
Component D	Elution Buffer	MC039	1mL

Procedures (for purification of GST fusion protein expressed in E.coli.):

Cell Lysates Preparation and Protein Precipitation:

1. Pellet cells from no more than 10 mL culture by spinning cells at 4000 x g for 5 minutes at RT.
2. Wash the cells once with 10 mL ice-cold Phosphate Buffered Saline (PBS) (Cat. #: CC008) and pellet cells by spinning at 4000 x g for 5 minutes at RT.
3. Resuspend in 1 mL Binding Buffer supplemented with 1 mg/mL lysozyme (Cat. #: PP012), 1 mM DTT (Cat. #: MC010), and Proteinase Inhibitor Cocktails (Cat. #: MP027). Transfer into 1.5 mL eppendorf tube.
4. Incubate the sample on ice for 30 minutes.
5. Sonicate the sample on ice to lyse cells (five times for 10 seconds each time with 5 seconds pauses between).
6. Centrifuge lysate at 10,000 x g for 15 minutes at 4°C. Collect supernatant.
7. Wash the Glutathione Sepharose 4B beads with 500 uL Binding Buffer. Centrifuge briefly to bring down the resin.
8. Carefully remove the Binding Buffer.
9. Add 1mL (or less) of the supernatant (from step 6) containing the GST fusion protein onto pre-washed Glutathione Sepharose 4B beads.
10. Incubate the resin on shaker for 30 minutes at 4°C.
11. Wash the Glutathione Sepharose 4B beads five times with 1 mL Washing Buffer (dilute to 1X with deionized water). Centrifuge briefly to bring down the beads after each wash.
12. After the last wash, carefully remove the Washing Buffer.

GST Fusion Protein Elution:

1. Add 30 uL of Imidazole elution solution to each sample and control resin.

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2. Incubate the sample with gentle shaking for 30 minutes at room temperature. Re-suspend the resin every 5 minutes by gentle pippetting.
3. 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.
4. For immediate use, store the supernatants at 2-8 °C. Store at –20 °C for long term storage.

Note:

- ✧ GST Fusion Protein Purification Kit contains sufficient reagents for 30 assays, which can purify proteins up to 10mL bacteria culture per assay.
- ✧ For culture in different size, increase or reduce the Glutathione Sepharose 4B and buffer volume propotionally.
- ✧ For different source of culture such as mammalian and plant cells, follow the desirable procedure to prepare the cell lysates.
- ✧ The Glutathione Sepharose 4B Resin is compatible with the most of the buffer systems that are used in biochemical application.

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