HIS-tagged Protein Purification Kit

Catalogue #: IPK004
Storage: 4—8 ºC
Size: Kit

Description:

The HIS Protein Purification Kit is designed for rapid purification of polyhistidine-tagged (His-tagged) recombinant 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. Up to 100μg of His-tagged protein can be purified in a standard assay. 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.

Kit Components:

Components	*Name*	*Cat#*	*Size*
Component A	Ni-NTA HIS Binding Resin	IT005C	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:

- ✧ Lysozyme (Cat. #: PP012)
- ✧ DTT (Cat. #: MC010)
- ✧ Phosphate Buffered Saline (PBS) (Cat. #: CC008)
- ✧ Proteinase Inhibitor Cocktails (Cat. #: MP027)

Procedures (for purification of HIS-tagged 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 Ni-NTA HIS Resin with 500 uL Binding Buffer. Centrifuge briefly to bring down the resin.

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8. Carefully remove the Binding Buffer.
9. Add 1mL (or less) of the supernatant (from step 6) containing the His-tagged protein onto pre-washed Ni-NTA HIS Resin.
10. Incubate the resin on shaker for 30 minutes at 4°C.
11. Wash the Ni-NTA HIS Resin five times with 1 mL Washing Buffer (dilute to 1X with deionized water). Centrifuge briefly to bring down the resin after each wash.
12. After the last wash, carefully remove the Washing Buffer.

HIS-tagged Protein Elution:

Elution with 0.1 M glycine HCl, pH 3.5 (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 resin for 30 seconds at 5,000 x g. Transfer the supernatants to a new tube containing Neutralization Buffer (1/10 volume of Elution Buffer).
4. Determine the protein concentration (Cat. #: MP022) and check the protein quality on SDS-PAGE gel following by Coomassie Blue Staining (Cat. #: MP002).

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

Elution with Imidazole (not provided)

1. Prepare Imidazole elution solution. Dilute 1M Imidazole (Cat. #: MC051) in Washing Buffer to 200 mM.
2. Add 300 uL of Imidazole 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 pippetting.
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.
5. For immediate use, store the supernatants at 2-8 °C. Store at –20 °C for long term storage.

Note:

- ✧ HIS-tagged Protein Purification Kit contains sufficient reagents for 30 assays, which can purify His-tagged proteins up to 10mL bacteria culture per assay.
- ✧ For culture in different size, increase or reduce the Ni-NTA HIS Binding Resin 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 Ni-NTA HIS Binding Resin is compatible with the most of the buffer systems that are used in biochemical application.

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