PRODUCT INFORMATION

Inclusion Body Protein Extraction Kit
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Components	Cat# IBP-2000
Cell Lysis Buffer I	30 ml
Cell Lysis Buffer II	20 ml
Deoxycholic Acid	1 ml
10x IB Solubilization Buffer I	5 ml
5x IB Solubilization Buffer II	100 ml
2x SDS Gel-loading Buffer	0.5 ml
DNase I	0.3 ml
Lysozyme	1 ml
KOH	5 ml
PMSF	0.3 ml

Procedure:

1 Centrifuge 1 liter of *E. coli* expressing the protein of interest at 5,000 x g for 15 minutes at 4°C in pre-weighed centrifuge bottles.

2 Remove the supernatant and determine the weight of the *E.coli* pellet. For each gram (wet weight) of *E. coli*, add 3 ml of Cell Lysis Buffer I. Resuspend the pellet by gentle vortexing or by stirring with a polished glass rod.

3 For each gram of *E. coli*, add 4 µl of protease inhibitor and then 80 µl of lysozyme. Stir the suspension for 20 minutes.

4 Stir continuously, and add 80 µl of deoxycholic acid per gram of *E. coli*.

5 Store the suspension at 37°C and stir it occasionally with a glass rod. When the lysate becomes viscous, add 20 µl of DNase I per gram of *E. coli*.

6 Store the lysate at room temperature until it is no longer viscous (approximately 30 minutes).

7 Purify and wash the inclusion bodies using Triton X-100. a) Centrifuge the cell lysate at 10,000 x g (12,000 rpm) for 15 minutes at 4°C. b) Decant the supernatant. Resuspend the pellet in 9 volumes of Cell Lysis Buffer II at 4°C. c) Store the suspension for 5 minutes at room temperature. d) Centrifuge at 10,000 x g (12,000 rpm) for 15 minutes at 4°C. e) Decant the supernatant and keep it aside for the next step. Resuspend the pellet in 100 µl H2O. f) Remove 10 µl samples from both the supernatant and the resuspended pellet. Mix each sample with 10 µl of 2x SDS Gel Loading Buffer for SDS-PAGE to determine which fraction contains the protein of interest. g) Proceed to Step 8 to solubilize the inclusion bodies.

8 Centrifuge the appropriate resuspended pellets from Step7 at 10,000 x g (12,000 rpm) for 15 minutes at 4°C in a microfuge, and suspend pellets in 1 ml of 1x Inclusion-Body Solubilization Buffer I (add 0.48 g urea and 10 µl PMSF).

9 Store the solution for 1 hour at room temperature.

10 Add this solution to 9 volumes of 1x Inclusion-body solubilization buffer II and incubate the mixture for 30 minutes at room temperature. Check if the pH is maintained at 10.7 by spotting small aliquots onto pH paper. If necessary, readjust the pH to 10.7 with 10 N KOH.

11 Adjust the pH of the solution to 8.0 with 12 M HCl, and store the adjusted solution for at least 30 minutes at room temperature.

12 Centrifuge the solution at 10,000 x g (12,000 rpm) for 15 minutes at room temperature.

13 Decant the supernatant and keep it aside for the next step. Resuspend the pellet in 100 µl of 1x SDS Gel-Loading Buffer.

14 Remove 10 µl samples from both the supernatant and the resuspended pellet. Mix with 10 µl of 2x SDS Gel-Loading Buffer. Analyze both samples by SDS-PAGE to determine the degree of solubilization.

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Note:

1 Perform step 2-4 at 4°C.

2 Each protein may require a different procedure, successfully refolding the denatured protein is highly dependent on the protocol, there are several techniques used to refold the denatured proteins, such as dilutions, dialysis, diafiltration and gel chromatography, here present the dilution technique, for other refolding protocols, please see the references.

3 Use the phase contrast microscope to detect if the *E.coli* is lysed thoroughly, for the intact cell will decrease the purity of the protein.

4 Though the protein exists in the inclusion body, there still have considerable soluble protein in some cases, so detect the supernatant.

5 Substitute 6M Guanidine hydrochloride for urea to get better results.

6 Use GSH/GSSG, cysteine/cystine, cysteamine/cystamine, DTT/GSSG, DTE/GSSG system to improve the renaturation yield. Chaperon, ligand, substrate and other small molecules can also increase the renaturation yield.

Reference:

1. Marston F.A. 1986. *Biochem. J.* 240:1-12.
2. Marston F.A. and Hartley D.L. 1990. *Methods Enzymol.* 182: 264-276.
3. Marston F.A., et al. 1984. *Bio/ Technology* 2:800-804.
4. Lilie H et al. 1998 Curr. Opin.. in Biotech. 9:497-501.

Caution:

Operate carefully with PMSF. It is extremely toxic and irritating to eyes and skin. Wear eye and hand protection to keep the solution containing PMSF away from skin and eyes. This product is for laboratory research use only.

Storage:

DNaseI, lysozyme and PMSF stored at -20 ^{o}C; others stored at RT.

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