Inclusion Body Protein Extraction Kit

Product information for BS686/BS687:

Introduction

The kit is designed for extraction of insoluble aggregates of the expressed proteins-Inclusion Body Proteins and the screening of E. coli clones that express recombinant proteins in inclusion bodies. The procedure is simple, rapid. The solublized proteins can be used for downstream experiments such as re-folding, SDS-Page Wester blots.2-D gel electrophoresis enzyme analysis. The kits can be used for 10 preps (10 x1 gram of wet weight E.coli) or 10 x 1 liters of E.coli culture(when OD_{600}.=1).

Components：

Cat#	BS686	BS687
Cell lysis buffer I	30 ml	90ml
Cell lysis buffer II	20 ml	60ml
Deoxycholic acid	1 ml	3ml
10x IB solubilization buffer. I	5 ml	15ml
5x IB solubilization buffer. II	100 ml	100ml X3
2x SDS gel-loading buffer.	0.5 ml	1.5ml
DNase I	0.3 ml	0.9ml
Lysozyme	1 ml	3ml
KOH	5 ml	15ml
Protease Inhibitor	0.3ml	1ml

Storage

DNaseI, lysozyme, 2x SDS gel-loading buffer and Protease Inhibitor stored at -20^0 C, for others store at room temperature.

Procedures:

1. Centrifuge 1 liter of the cell culture of *E. coli* expressing the protein of interest at 5,000*g* for 15 minutes at 4°C in preweighed 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 protease inhibitor and then 80µl lysozyme. Stir the suspension at 37°C for 20 minutes.
4. Stirring continuously, add 80ul 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 ul DNase I per gram of *E. coli*.
6. Store the lysate at room temperature until it is no longer viscous (approx. 30 minutes).
7. Purify and wash the inclusion bodies.
    a. Centrifuge the cell lysate at 12,000rpm for 15 minutes at 4°C in a microfuge tube;
    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 12000rpm for 15 minutes at 4°C in a microfuge tube.
    e. Decant the supernatant and keep it aside for the next step. Resuspend the pellet in 100 ul of H_2O.
    f. Remove 10 ul samples of the supernatant and of the resuspended pellet. Mix each sample with 10µl of 2x SDS gel loading buffer for SDS-PAGE to determine which fraction contains the protein of interest.
    g. Proceed with Step 8 to solubilize the inclusion bodies.

地址: 上海市松江区香闵路 698 号 Add: 698 Xiang Min Road SongJiang Shanghai China
电话/Tel: 021-37772436; 800-820-1016;400-821-0268 传真/Fax: 86-21-37772170
邮箱/Email: protein@sangon.com 网址/Web: www.sangon.com

8. Centrifuge the appropriate resuspended pellets from Step 7 at 12000rpm for 15 minutes at 4°C in a microfuge tube, and suspend pellets in 1ml 1x Inclusion-body solubilization buffer I (before use,add 0.48g urea and 10ul of 0.1M PMSF or Protease Inhibitor(100x)).
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 that 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 12000rpm for 15 minutes at room temperature in a microfuge tube.
13. Decant the supernatant and keep it aside for the next step. Resuspend the pellet in 100 ul of 1x SDS gel-loading buffer.
14. Remove 10ul samples of the supernatant and of 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

Notes :

1. Perform step 1-2 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 and gel chromatography, for other refolding protocols, please see the references. For each protein 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.
3. Though the protein exists in the inclusion body, there still have considerable soluble protein in some cases, so detect the supernatant.
4. Substitute 6M Guanidine hydrochloride for urea to get better results.
   Use GSH/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.

Cautions:

Operate carefully for Protease Inhibitor is harmful to health, it is extremely toxic & is irritating to eyes & skin. Wear eye & hand protection to keep the solution containing Protease Inhibitor away from skin and eyes. This product is for laboratory research use only.