Gpure Inclusion Body Purification Kit

INTRODUCTION

Heterologus expression of foreign genes in *E coli.* can lead to intracellular accumulation of recombinant protein or to secretion and accumulation in the periplasmic space. However recombinant protein accumulated intracellularly is frequently laid down in the form of Inclusion Bodies (IB), insoluble aggregates of misfolded protein lacking biological activity. Typically, 70-80% of proteins produced by recombinant techniques in *E. coli* form inclusion bodies. A solution dependent purification kit allow researcher to purify IB without using any sort of chromatography techniques.

FEATURES

- The kit provides essential solutions for inclusion body purification only. The kit includes solutions and protocols for use with either acidic or basic proteins.
- Purified IB can be further used in affinity chromatography.
- Analyse 1.5 mL of bacterial cell culture yielding 2–50 ug of protein or 100 ml bacterial cell culture to obtain up to 12 mg of protein.

APPLICATION

- Mass Spectrometry
- SDS-PAGE
- Re-folding Experiments

PROTOCOL

1. Suspend the cell pellet in appropriate amount of GLyseB buffer as prescribed in the respective product datasheet.
2. The cells can be lysed following recommended protocol (Please consult the product datasheet). (In case of inclusion body purification complete lysis of *E.coli* cell will ensure better quality of purified IB, therefore use of sonication / freeze-thaw technique is advised in some cases)
3. Centrifuge cell lysate for approximately 20 min at 10,000 rpm, 4°C.
4. Resuspend the cell pellet in 0.5X or 1X Reagent A and incubate for 10- 30 min (RT).
5. Centrifuge at 10,000 rpm for 20 minutes.
6. Repeat the step 4 -6
7. Wash the inclusion body pellets in a small volume of Reagent B and stir for 10 minutes on bench. This step solubilize membranes and membrane proteins. A short sonication (3 x 10 seconds) is very helpful during each wash step. It helps to resuspend all the inclusion bodies within in short incubation periods.
8. Centrifugation as in step 5.
9. Repeat the step 7-8 for another two rounds.
10. Wash the inclusion body pellets in a small volume of Reagent C and stir for 30 minutes on bench. Some DTT up to 50 mM, should be used in all subsequent steps to keep disulphides reduced, this is important for all proteins.
11. Centrifugation as in step 5.
12. Repeat the step 10 -11 one more time.
13. Subject the pellet with 0.5 M NaCl wash.
14. Solubilize the purified inclusion bodies into 6M Guanidine HCl (GnCl-HCl) or 8M urea with appropriate buffer and DTT/ β-Me*.
15. Dialyse the sample against appropriate buffer.
16. Analyze the sample on SDS-PAGE.

** The optimal concentration of reducing agent must be determined experimentally for each individual protein. Some inclusion bodies are very difficult to solubilise and you might want to leave them to dissolve over-night. Heating in a bath (37-50˚C) may facilitate the pellet dissolving.*