INSTRUCTIONS

Inclusion Body Solubilization Reagent

78115

0868.2

Number	Description
78115	**Inclusion Body Solubilization Reagent**, 100mL
	Storage: Upon receipt store at room temperature.

Introduction

The Thermo Scientific Inclusion Body Solubilization Reagent recovers insoluble proteins expressed in inclusion bodies. Typically, 70-80% of proteins produced by recombinant techniques in *E. coli* form inclusion bodies (i.e., protein aggregates). A proprietary denaturant in this reagent is the most effective means for solubilizing aggregated proteins. Additional components, such as reducing and chelating agents, are compatible. This useful method is an essential before proceeding with protein refolding.

Inclusion bodies typically form when proteins are overexpressed, allowing high levels of expression. Inclusion bodies are easily separated from a large proportion of bacterial cytoplasmic proteins by centrifugation using Thermo Scientific B-PER Bacterial Protein Extraction Reagent.[1]

Important Product Information

- **Protein refolding:** After solubilization, the protein will be denatured. Therefore, protein refolding is necessary for activity. Several published methods for protein refolding are available.[2-4] An example refolding protocol is included in these instructions. For best results, empirically determine the optimal refolding protocol for each specific protein.
- **Compatibility:** The denaturant included in the Inclusion Body Solubilization Reagent precipitates in SDS-PAGE sample buffer. The denaturant can be removed by dialysis before performing SDS-PAGE analysis. The Inclusion Body Solubilization Reagent is, however, compatible with Thermo Scientific Coomassie Plus (Bradford) Protein Assay Kit (Product No. 23236).

Procedure for Protein Solubilization

1. Purify inclusion bodies using B-PER™ Bacterial Protein Extraction Reagent (Product No. 78248) or by other methods. If desired, analyze purity by SDS-PAGE before solubilization. Inclusion body purity does not affect solubilization efficiency; however, if subsequent refolding procedure is desired, > 90% purity is optimal.
2. Estimate the amount of inclusion body prep by subtracting the weight of the centrifuge tube from the total weight. Use 8mL of the Inclusion Body Solubilization Reagent per gram of wet inclusion body pellet. Use more reagent as needed.
3. Suspend the pellet in an appropriate amount of Inclusion body Solubilization Reagent by either vigorous vortex mixing or by pipetting until the suspension is homogenous. Shake the suspension for 30 minutes.
4. Remove the cell debris by centrifugation at 27,000 × *g* (15,000 rpm for Beckman JA20 rotor) for 15 minutes.
5. Collect the supernatant, which contains the solubilized protein. If a protein assay is desired, use the Coomassie Plus™ (Bradford) Protein Assay Kit (Product No. 23236). For SDS-PAGE analysis, remove the denaturant by dialysis.

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Protein Refolding Using Dialysis Method

A. Additional Materials required

- B-PER Bacterial Protein Extraction Reagent (for inclusion body purification)
- Dithiothreitol (DTT, Product No. 20290)
- Urea
- Thermo Scientific Slide-A-Lyzer Dialysis Cassette: Dialysis (Product. No. 66810)

B. Protocol

1. Purify inclusion bodies using B-PER Bacterial Protein Extraction Reagent and solubilize inclusion body protein using Inclusion Body Solubilization Reagent. If disulfide bonds are involved in refolding, add DTT to 5mM (final) to the reagent during solubilization.
2. Prepare 1L of 6M urea in a 3.5L beaker.
3. Use an 18-gauge needle and a 10mL syringe to transfer 8mL of the Inclusion Body Protein Solution to a 3-12mL Slide-A-Lyzer™ Cassette. Dialyze the inclusion body protein against 6M urea for 6 hours.
4. Add 250mL of 25mM Tris•HCl (pH 7.5) to the beaker every 6-12 hours.
5. Once the volume reaches 3L, replace the dialysis solution with 2L of 25mM Tris•HCl (pH 7.5) and 150mM NaCl. Dialyze for another 6 hours. To maintain protein stability, perform the dialysis in a cold room (4-8°C). Precipitation might form during dialysis; however, some protein remains in solution.
6. Recover sample from the dialysis cassette. Remove any visible insoluble material from the sample by centrifugation. Determine the folding status of the soluble protein by activity assay or other method.

Related Thermo Scientific Products

78248	**B-PER Bacterial Protein Extraction Reagent,** 500mL
23236	**Coomassie Plus™ Protein Assay Reagent,** 950mL
20290	**DTT, Cleland's Reagent, Dithiothreitol,** 5g
29700	**Urea, Sequanal Grade,** 1kg
66810	**Slide-A-Lyzer Dialysis Cassette, 10K MWCO, 3-12mL capacity,** 8/pkg.
89867	**Pierce Protein Refolding Kit**

Cited References

1. Chu, R., *et al.* (1998). Recombinant protein extraction from *E. coli* using B-PER™ Bacterial Protein Extraction Reagent. *Previews* **2(1):**12-13.
2. Mukhopadhyay, A. (1997). Inclusion bodies and purification of protein in biologically active forms *Adv. Biochem Eng Biotechnol* **56:**61-109.
3. Rudolph, R. and Lilie, H. (1996). *In vitro* folding of inclusion body proteins. *FASEB J* **10:**49-56.
4. Marston, F.A.O. and Hartley, D.L. (1990). Solubilization of protein aggregates. *Meth Enzymol* **182:**264-76.

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