PROMETHEUS™
Protein Biology Products

Instruction Manual

Bacterial Protein Extraction Buffer Catalog No.: 18-400, 18-401, 18-402 & 18-403

Introduction:
Our Bacterial Protein Extraction Buffer is designed for gentle and efficient extraction of soluble proteins and inclusion bodies from bacteria (e.g. *E. coli*), which doesn't require lysis with mechanical disruption. Bacteria possess a rigid cell wall composed of peptidoglycans that must be disrupted for cellular proteins to be released. Our bacterial protein extraction buffer contains a proprietary nonionic detergent with an organic buffer system and other agents, which does not require sonication or any other mechanical steps to lyse the bacterial cells. The unique composition offers adaptability for different applications and which excludes exogenous contamination of the recombinant protein by the lysis reagent. In addition, it offers several folds increase in the yield of soluble protein when compared with other bacterial lysis reagent. The Bacterial Protein Extraction Kit is supplied with premade stabilized preparations of Lysozyme and Nuclease enzymes to improve the extraction efficiency of large molecular weight (> 70kDa) proteins and proteins expressed in inclusion bodies and removal of DNA/RNA released during the bacterial lysis. Depending on the particular application, additional components, such as protease inhibitor cocktail, reducing agents and chelating agents may be added to the solution. The solution may be used for both soluble protein extraction and inclusion body purification from bacterial cell lysates.

The method involves the bacterial cell pellets to be incubated with the supplied extraction buffer along with lysozyme and nuclease enzymes, that help disrupt cell walls and digest nucleic acids respectively and centrifuged to separate cell debris and insoluble protein (e.g., inclusion bodies). The supernatant contains the soluble protein fraction, which can be further purified or directly analyzed, for example by SDS-PAGE. The protein yields obtained with our Bacterial Protein Extraction Buffer are much higher than those obtained from standard sonication methods. Bacteria often overexpress recombinant proteins and form inclusion bodies, which are insoluble aggregates of misfolded protein. Centrifugation separates inclusion bodies from soluble proteins; however, Lysozyme is required for purification of inclusion bodies. Lysozyme supplied with the kit significantly improves inclusion body purity by digesting the cell debris. The bacterial cell lysates prepared with our Bacterial Protein Extraction Solution are in native state and can be used for assaying biological activities and compatible with BCA, Bradford Protein Assays, Electrophoresis, Western Blots and other downstream applications.

Kit Components:

Item Name	Cat. No. 18-400	Cat. No. 18-401	Cat. No. 18-402	Cat. No. 18-403	Storage Condition*
Bacterial Protein Extraction Buffer	250 ml	500 ml	125 ml	250 ml	4 °C
Lysozyme (40mg/ml, 40K Units/mg)	N/A	N/A	0.5 ml	1 ml	-20 °C
Nuclease (2,500 Units/ml DNase & 1,200 Units/ml RNase)	N/A	N/A	0.5 ml	1 ml	-20 °C

**The individual buffer and complete kit with enzymes are shipped at ambient temperature. Upon receipt, store the individual kit components as marked. The kit components are stable for one year, if stored and used as recommended.*

Items Needed (Not Supplied with the Kit):
- Appropriate Bacterial Cell culture
- Centrifuge
- Incubator
- Laboratory consumables

Important Notes on the Product
- The Bacterial Protein Extraction Buffer extracts proteins from freshly prepared or frozen bacterial cells. Extraction is typically more effective with frozen cells. The amount of reagent required depends on the amount of cell pellet size.
- If desired, Protease inhibitors, EDTA Free (Genesee Cat. No. 18-425), salts, and reducing agents (e.g. DTT can be added directly to the extraction buffer. Chelating agents, e.g. EDTA can be used with the buffer, if the presence of divalent ion is not necessary for downstream application.
- Add lysozyme and Nuclease to extraction buffer for the most efficient extraction; however, the extraction of some over-expressed proteins does not require the addition of lysozyme. If the addition of lysozyme and Nuclease might interfere with the downstream applications, the use of these enzymes should be excluded.

- The bacterial protein extraction buffer effectively extracts soluble proteins from several common bacterial host strains, and if lysis is inefficient for a particular bacterial strain, freeze cells before extraction.
- Whole cell lysates prepared with bacterial protein extraction are compatible with BCA (Cat. No. 18-440) and Bradford Coomassie (Cat. No. 18-442) Protein Assays

Procedures for Protein Extraction from Bacteria:

Extracting of Soluble Proteins Fraction

1. Use fresh bacterial cells or frozen at –80ºC, however, protein extraction is typically more effective in frozen cells. Pellet bacterial cells from 1.5 ml bacterial culture (OD600 1.5 – 3.0) at 10,000 rpm (5,000 x g) for 10 minutes in a microcentrifuge. **NOTE**: For large volume (50-250 ml) bacterial culture, pellet cells by centrifugation at 3,500 g for 10 minutes.
2. Remove all the media by aspiration. The cells can either be used fresh or frozen at –80ºC. Protein extraction is typically more effective with frozen cells.
3. Mix the Lysozyme and Nuclease tubes (supplied with Kits 18-402 & 18-403) by gentle vortexing and add 2µl of lysozyme and 2µl of Nuclease per ml of Bacterial Protein Extraction Buffer and add EDTA Protease inhibitors (optional).
4. Resuspend the bacterial pellet in 125µl of Bacterial Protein Extraction Buffer (5 volumes of extraction buffer per 25µl wet pellet) by pipetting up and down or vortex for ~ 30 sec, until the cell suspension is homogeneous. **NOTE***:* For bacterial pellet from a 50 ml of culture, resuspend in 3-4 ml of Extraction Buffer and if the pellet was from 250 ml of culture, use 15-20 ml Extraction Buffer.
5. Incubate the suspension at 37°C for 30-45 minutes and at the end of incubation, vortex the tube a few times for 30 sec each to complete the lysis.
6. Centrifuge the lysate at 15,000 x g for 5 minutes to separate the soluble and insoluble protein fractions. The soluble protein is in the supernatant. **NOTE**: For larger volumes, separation of soluble and insoluble protein fractions can be achieved by centrifugation at 27,000 x g for 15 minutes.
7. Transfer the supernatant to a clean tube and resuspend the insoluble fraction in 300µl Extraction Buffer. Use 10µl each of the soluble and insoluble fraction for SDS-PAGE and/or Western blotting to determine the solubility of the recombinant protein of interest. **NOTE***:* In larger scale preparations, resuspend the insoluble fraction in twice the volume of Extraction Buffer used in Step 3.

For Inclusion Body Purification

If a large percentage of over-expressed protein remains in the pellet, the protein of interest might be expressed in inclusion bodies. To overcome this issue, either use an inclusion body solubilization buffer or modify the expression conditions to minimize inclusion body formation or use this alternate protocol below, which may or may to work hundred percent (not guaranteed).

1. Resuspend the bacterial pellet from step 2 in the same volume of Extraction Buffer as used in step 4 soluble fraction steps.
2. Add higher concentration of lysozyme to the resuspended pellet to a final concentration of 400µg/ml. Vortex for 1 minute and incubate at room temperature for 5 minutes.
3. Add 6x volume of 1:20 diluted Extraction Buffer to the suspension and vortex for 1 minute.
4. Collect inclusion bodies by centrifugation at 13,000 rpm in a microcentrifuge for 10 minutes. Remove the supernatant with a pipette. **NOTE**: For larger volumes, separation of inclusion bodies can be achieved by centrifugation at 27,000 g for 15 minutes.
5. Step 4 and 5 may be repeated for multiple rounds of purification if necessary.
6. Resuspend the pellet of the purified inclusion bodies in the buffer of your choice.

Related Products

1. **BCA Protein Assay Kit (Cat. No. 18-440 & 18-441)**
   For protein estimation and is also compatible with proteins extracted with our Protein Extraction Buffers
2. **Protein Loading Buffer [2X], Cat. No. 20-309**
   Non-reducing ready to use buffer for loading protein samples on to the gel.
3. **Protein Loading Buffer [2X], Cat. No. 20-310**
   Reducing ready to use buffer for loading protein samples on to the gel.