Product Manual

5X Bacterial Protein Extraction Reagent (Phosphate)

Catalog Number

AKR-181	50 mL of 5X Concentrate in Phosphate-based buffer, pH 7.5

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Bacteria are widely used for recombinant protein expression. However, mechanical cell disruption methods (e.g. sonication, steel beads, high-shear) are often time-consuming or limited by sample volume, sample number, disruption efficiency, protein functionality, and total yield.

The Cell Biolabs Bacterial Protein Extraction Reagent contains a gentle, nonionic detergent formulation which quickly extracts functional, recombinant protein from *E. coli* without mechanical disruption. Other benefits include the following:

- Designed specifically for mild extraction of functional, recombinant proteins from *E. coli* without mechanical disruption
- Produces high yields in a single-step, 15-minute protein extraction (entire procedure takes less than 30 minutes)
- Designed for small-scale or large-scale protein extraction
- Protease inhibitors, reducing agents, Lysozyme and/or DNase I are easily added to improve lysis/recovery
- Compatible with 6xHis and GST affinity purification systems
- Phosphate buffer formulation for direct labeling and conjugation
- Recovered lysates are compatible with SDS-PAGE, BCA and Bradford Protein Assays
- Can be used with frozen or recently prepared *E. coli* pellets

Related Products

1. AKR-103: PhosphoBLOCKER™ Blocking Reagent
2. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation Kit
3. AKR-180: Bacterial Protein Extraction Reagent (Tris)
4. AKR-190: 5X RIPA Buffer

Storage

Store at room temperature for up to 1 year from date of receipt.

Preparation of Reagent

- 1X Bacterial Protein Extraction Reagent: Dilute the 5X Bacterial Protein Extraction Reagent to 1X with deionized water. Stir to homogeneity. Store at room temperature.

Note: If desired, protease inhibitors, reducing agents, Lysozyme and/or DNase I may be added to the diluted 1X solution.

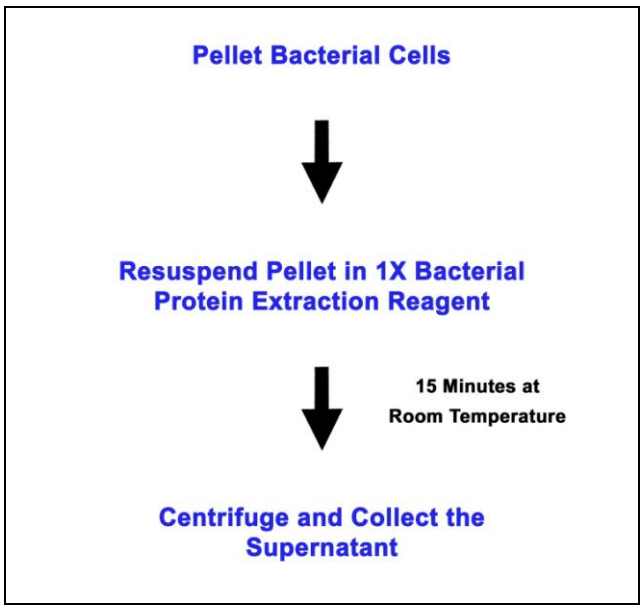

Figure 1: Bacterial Protein Extraction Flow Chart

Assay Protocol

1. Pellet bacterial cells by centrifugation at 5000 x *g* for 10 minutes.
2. Aspirate the media from the pellet.
3. Resuspend the cell pellet with 1X Bacterial Protein Extraction Reagent (see Preparation of Reagent Section). Add 4 mL per gram of cell pellet. Triturate cells up and down until the solution is homogeneous.
4. Incubate 15 minutes at room temperature.
5. Centrifuge the lysate 12000 x *g* for 10 minutes.
6. Carefully transfer the supernatant to another tube, making sure not to disturb the insoluble pellet.

   Note: If desired recombinant protein is not soluble (forming inclusion bodies), adjust the expression conditions or use an inclusion body solubilization reagent (such as #AKR-110 for GST fusion proteins).

Example of Results

The following figure demonstrates typical results with the Bacterial Protein Extraction Reagent. One should use the data below for reference only. This data should not be used to interpret actual results.

Figure 2: Extraction of His-RFP fusion protein. His-RFP expression in BL21 was induced with 1 mM IPTG at 37ºC for 4 hrs. Cells were pelleted (left), then resuspended in Bacterial Protein Extraction Reagent for 15 minutes, according to the assay protocol. The supernatant (right) containing the extracted His-RFP was collected from the insoluble post-extraction pellet (center).

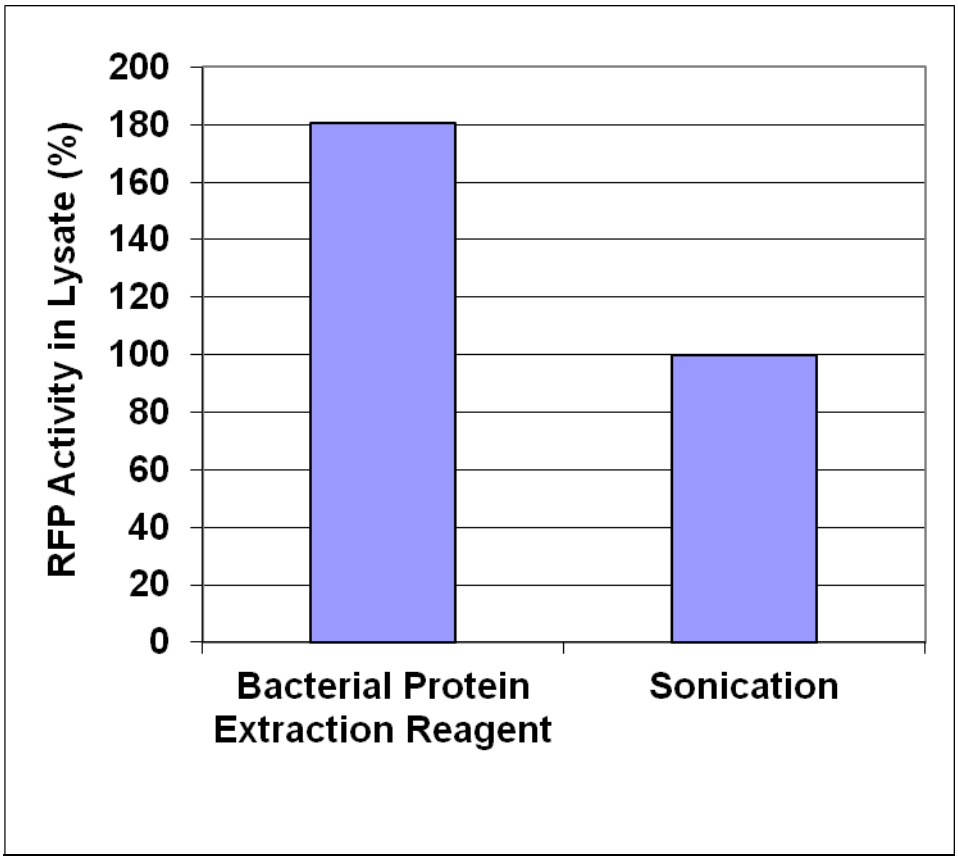

Figure 3: Comparison of Sonication with Bacterial Protein Extraction Reagent. Pelleted His-RFP cells were sonicated in PBS/Triton X-100 or resuspended in Bacterial Protein Extraction Reagent for 15 minutes. Lysates were then collected and tested for RFP fluorescence on a 96-well fluorometer.

Recent Product Citation

Lee, J. et al. (2018). Structural Characteristics of Pneumolysin and Its Domains in a Biomimetic Solution. *ACS Omega*. **3**(8):9453-9461. doi: 10.1021/acsomega.8b01212.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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