

EZLys™ Bacterial Protein Extraction Reagent

CATALOG #: 8001-100 100 ml
8001-500 500 ml

LOT #: _____

STORAGE: Store at 2-8 °C (**Note:** If precipitation is present, warm to room temperature, mix before use.)

SHELF LIFE: 1 year under proper storage conditions

DESCRIPTION:

A proprietary formulation prepared in a 50 mM Tris pH8.0 buffer and designed for gentle disruption of cell membrane to extract proteins. It provides a simple, rapid, low cost method for releasing expressed target protein for preparation, purification, or other applications. The EZLys™ reagent is an optically transparent, non-denaturing formulation which can be easily removed by dialysis. It is also useful for the preparation of high purity inclusion bodies for subsequent purification. This reagent does not interfere with typical protein immobilization or cross linking and is suitable for use in the BCA or Bradford protein assays. Ideal pH range is pH 5 -10. The 100 ml and 500 ml reagent are sufficient for protein extraction from 25 g and 125 g of cell paste.

ADVANTAGES OF USING THE REAGENT:

- 1) Complete cell lysis without mechanical stress which is tedious and can damage proteins.
- 2) The extracted proteins are in native, non-denatured form.
- 3) The reagent does not interfere with down-stream functional testing or protein assays.
- 4) The reagent can easily be removed by dialysis or spin-column buffer exchange.

SUGGESTED PROTOCOL:

A. Soluble Protein Extraction:

- 1) Collect cells by centrifugation at 10,000g x 10 minutes in a pre-weighed centrifuge tube. For a small scale preparation (e.g., ≤ 1.5 ml), centrifugation can be performed in an eppendorf tube 13000-16000g x 10 min. Remove as much liquid as possible. Determine wet weight of the cell pellet.
- 2) Resuspend the pellet in minimum 4 ml of the EZLys reagent (prewarmed to room temperature) per 1g of wet cell paste by pipetting and/or gentle vortexing.

Optional: a) Addition of protease inhibitors is recommended at this time;

b) Addition of Turbonuclease (10-100 U/g wet cell paste) and Lysozyme (5 KU/g wet cell paste) may reduce viscosity and improve protein extraction.

- 3) Incubate the cell suspension by shaking or slow mixing for 5 minutes or longer.
- 4) Remove insoluble debris by centrifugation at 16,000g x 20 min at 4°C. The supernatant containing the soluble proteins can now be analyzed or further purified such as using a Ni-NTA column or stored at -20°C for future use. (**Note:** The pellet contains inclusion bodies. If desired, save the pellet for inclusion body purification.)

B. Inclusion Body Purification:

- 1) Resuspend the pellet (from Step A4) in the same volume of EZLys reagent which was used to resuspend the cell pellet (Step A2) by pipetting up and down and vortexing to obtain an even suspension. The pellet needs to be completely resuspended.
- 2) Add lysozyme to a final concentration of 1 KU/ml, mix gently and incubate at room temperature for 10 min.
- 3) Centrifuge at 5000g x 15 min. at room temperature. Carefully remove supernatant.
- 4) Resuspend the pellet with 1:10 diluted EZLys using about 8 ml per gram of original wet cell pellet and mix by vortex; centrifuge at 5000g x 15 min. at room temperature to collect the inclusion body.
- 5) Repeat the step 4) above two more times.
- 6) Take aliquot for SDS-PAGE analysis. Pellet the inclusion bodies at 16000g x 15 min at 4 °C. Remove supernatant. The purified inclusion bodies can be used immediately, or stored at -80 °C for future use.

RELATED PRODUCTS:

- Antibiotics
- Cell Fractionation kits
- Protein Extraction Kits
- Cell Lysis Agents
- Detergents
- Refolding Buffers
- Phosphatase Inhibitors
- Phosphatase Inhibitor Cocktails
- Protease Inhibitors
- Protease Inhibitor Cocktails
- Tag Antibodies (HA, His, cMyc, GST, GFP, etc.)
- Cellular Marker Antibodies
- Loading Control Antibodies
- Secondary Antibodies
- Protein Quantitation Kit
- SDS-PAGE Loading Buffer
- Western Blot Substrate Kit

OTHER FEATURED PRODUCTS:

- Multi-color Fluorescent Proteins
- Stem Cell Research Products
- Apoptosis Assay Kits & Reagents
- Metabolism Assay Kits
- Obesity and Diabetes Research Products
- Protein Purification Tools
- Cytokines & Growth Factors
- Antibodies

FOR RESEARCH USE ONLY! Not to be used on humans.