USER GUIDE: EBL Fast-B Lysis 10X Reagent/Kit

About the Kits:

PFL-010010	EBL Fast-B Lysis 10X Reagent	10 ml
PFL-010100	EBL Fast-B Lysis 10X Reagent	100 ml
PFL-020040	EBL Fast-B Lysis Kit:	
	EBL Fast-B Lysis 10X Reagent	40 ml
	Nucleic acid-Cut (C300112)	10 KU
	Lysozyme (C3002040)	600 KU

Description:

EBL Fast-B Lysis Reagent is formulated for gentle disruption of *E. coli* cell wall to liberate active proteins, and is compatible with any protein purification systems, including but not limited to affinity, ion exchange, hydrophobic chromatography. It provides a simple, rapid, low-cost alternative to mechanical methods such as French Press or sonication for releasing expressed target protein in preparation for purification or other applications. EBL Fast-B Lysis reagent provides combinations zwitterionic detergents that are capable of cell wall perforation without denaturing protein. EBL Fast-B Lysis 10X reagent is a concentrated formulation of the proprietary detergents employed in Fast-B Lysis without buffer components, allowing user-defined dilution.

Features and Benefits:

- The 10X concentrated solution is compatible with any extraction buffer.
- More efficient and faster than ultrasonication.
- Fully compatible with affinity and other purification methods.
- After soluble protein extraction, inclusion bodies can be isolated for subsequent solubilization.
- Non-denaturing cell lysis preserves protein structure and function.

※ **Storage**: Store EBL Fast-B Lysis 10X Reagent/Kit at room temperature. Nucleic acid-Cut nuclease preparation is stable for 2 years when stored at -20°C. DO NOT store at -70°C because freezing Nucleic acid-Out nuclease results in loss of activity. Store Lysozyme reagent at –20°C.

Applications:

1. Lytic for both Gram positive and negative bacteria
2. Also suitable for culture cells.

Quick-start:

The following examples illustrate the use of EBL Fast-B Lysis 10X Reagent/Kit, and are intended as guidelines only. Optimal conditions will vary from application to application, depending on buffer compositions, and sample properties.

Soluble Fraction in E. coli lysates

A small scale trial extraction should be performed to determine the fraction in which the protein of interest will be found. The optimal detergent concentration for extraction of the protein of interest can also be determined at this time. It is recommended that 5, 10, and 20-fold dilutions (into the desired buffer) of the EBL Fast-B Lysis 10X Reagent concentration be tested. If the optimal detergent concentration has already been determined, proceed to the large scale extraction procedure.

NOTE: This trial is not always needed. For saving times and reagents, you can go to the large scale extraction procedure.

1. Dilute the concentrated EBL Fast-B Lysis 10X Reagent (at the various test concentrations) into the desired buffer system.

2. Harvest cells from liquid culture and resuspend the cell pellet at room temperature with EBL Fast-B Lysis reagent by pipetting or gentle vortexing (using 1 ml reagent per 0.2 gram of wet cell paste from 20 ml original culture medium). There are no adverse effects using large amount of EBL Fast-B Lysis Reagent.
   Optional: To reduce viscosity and improve protein extraction efficiency of the lysate, add 2.5 µl (25 units) Nucleic acid-Cut Nuclease and 1 KU lysozyme solution per 1 ml Fast-B Lysis reagent used for resuspension. Add protease inhibitors, if needed.

3. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10-20 min at room temperature to ensure full extraction of the soluble proteins.

4. Centrifuge the cell lysate at full speed for 10 minutes at 4°C to pellet any insoluble material.

5. Carefully remove the soluble protein fraction from the cell debris. Analyze the supernatant by SDS-PAGE and/or Western blot to determine the protein of interest. For SDS-PAGE, it is recommended that 5-15 µl of each sample be

applied to the gel.

Note: If the protein of interest is not found in the soluble portion, it has likely formed inclusion bodies. For the purification/solubilization of inclusion bodies see the Inclusion Body Purification Procedure.

6. The remaining soluble extract can be loaded directly onto any EBL purification resin. To maintain clarified extractions freeze them at –20°C until needed.

Inclusion Body Purification

1. Resuspend the cell pellet from the first extraction in an equal volume of the diluted EBL Fast-B Lysis Reagent solution that was used for the extraction. Vortex for 1-2 minutes to completely resuspend the cell debris. Add lysozyme to a final concentration of 1-2 KU/ ml.

2. Incubate at room temperature for 5-10 minutes to allow the lysozyme to fragment the cell wall.

3. Centrifuge at full speed for 10 minutes at 4°C to pellet the cell debris again. Save the supernatant for analysis.

4. Resuspend the pellet in an equal volume of EBL Fast-B Lysis 10X Reagent diluted 100-fold and vortex to completely resuspend any remaining in soluble material.

5. Centrifuge at full speed for 10 minutes at 4°C to pellet the cell debris. Save the supernatant for analysis. Steps 4 and 5 may be repeated 3-5 times to completely remove any remaining soluble proteins and cell wall material from the inclusion bodies. This wash step should be optimized for the specific protein of interest.

6. Resuspend the washed inclusion bodies pellet from step 5 in an equal volume of deionized water or a buffer of choice.

7. Analyze all of the saved supernatants and the insoluble fraction by SDS-PAGE and/or Western blot. For SDS-PAGE, it is recommended that 5-15 µl of each sample be applied to the gel.

8. Store all proteins at -20/-80 °C until needed.