Product information

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Bacterial Protein Lysis Buffer

Catalog #: BSP023
Size: 25ml
Storage: -20°C*

*: Product will be shipped with ice pack. Check storage conditions. Products have one year expiration from time of purchase.

Product Description:

Bacterial Protein Lysis Buffer enables mild extraction of proteins from bacteria (E. coli) without the need for mechanical disruption. The reagent may be used for soluble protein extraction and inclusion body purgation from bacterial cell lysates. The kit is supplied with lysozyme and DNase I to improve extraction efficiency of large (>60 kDa) molecular weight proteins and proteins expressed in inclusion bodies. Furthermore, the kit is supplied with PMSF and DTT to improve protein stability and integrity. Depending on the particular application, additional components, such as protease inhibitors, salts, phosphatase inhibitors and chelating agents may be added to the buffer (add chelating agents only when using the buffer without enzymes). Soluble protein is suitable for immunoprecipitation, immunoassay, SDS-PAGE, and affinity purification. 25 ml is sufficient for 20 extractions of 100 mg wet weight bacterial sample.

Features:

- Can be used to recover soluble protein by shaking for 20 minutes and pelleting cell debris, or can recover insoluble recombinant protein from the pellet using an optimized procedure.
- Suitable for any scale of protein extraction.
- Completely compatible with addition of protease inhibitor cocktails. Resulting protein extract can be used in protein assays, typical affinity purification methods (e.g., GST, 6xHis) and other applications.

Composition:

Lysis Buffer	25 ml
Lysozyme	100 µl
DNase I	100 µl
PMSF	300 µl
DTT(1M)	30 µl

Storage:

Upon receipt, store Lysis Buffer at ambient temperature and store Lysozyme, PMSF, DTT (1M) and DNase I at -20°C. These products have one year expiration from time of purchase.

Procedure for Extracting Protein from Bacteria

1. Pellet bacterial cells by centrifugation at 5,000 × g for 10 minutes.
2. Optional: Add 2 µl of lysozyme and 2 µl of DNase I per 1 ml Protein Lysis buffer. Add EDTA-free protease inhibitors.
3. Add 0.5 ml of Protein Lysis buffer per 0.1 g of cell pellet (before use add 1 µ DTT and 10 µl PMSF per 1 ml Lysis buffer). Pipette the suspension up and down until it is homogeneous.
4. At room temperature, agitate (using a platform mixer) at 280 rpm for 20 minutes.
5. Centrifuge lysate at 15,000 × g for 5 minutes to separate soluble proteins from the insoluble proteins.

NOTE: Typically > 80% of the soluble protein is recovered by this extraction. To further increase yield, steps 3-5 may be repeated.

6. Suspend pellet (insoluble fraction) in 0.25 ml Protein Lysis buffer, use 10-20 µl of the soluble and insoluble fractions for SDS-PAGE or Western blot to determine protein solubility. If purification of inclusion bodies is desired, proceed to the next step.

7. Add 1 µl of Lysozyme and 0.5 µl of DNase I into 0.25 ml pellet suspension and incubate at room temperature at 30 minutes. After 30 minutes, add 1 ml of 1:10 diluted Protein Lysis buffer to the suspension and mix by vortexing.

8. Centrifuge tube at 15,000 x g for 15 minutes. Resuspend pellet in 1 ml of 1:10 diluted Protein Lysis buffer. Repeat this step two more times.

9. Dissolve the purified inclusion bodies in denaturing agents and proceed to further refolding or purification procedures.

Additional Notes:

1. Extractions can be done from recently prepared cells and frozen cells. Extraction is typically more effective with frozen cells. The amount of reagent required depends on the amount of cell paste.

2. If desired, add EDTA-free protease inhibitors (BS380) and salts directly to the Lysis buffer. Chelating agents such as EDTA can be used after cells have been lysed, but may destroy Ni-NTA Resin and affect the activity of DNase I.

3. Add lysozyme and DNase I to Protein Lysis buffer for the most efficient extraction. However, it should be noted that the extraction of some over-expressed proteins does not require the addition of lysozyme. If the addition of lysozyme and DNase I interferes with downstream applications, the use of these enzymes may be omitted.

4. Some over-expressed proteins can be insoluble, misfolded or expressed in inclusion bodies. The best way to obtain soluble protein is to adjust the expression conditions; however, if this is not possible, Inclusion Body Solubilization reagents may be used.

5. Although this buffer effectively extracts soluble proteins from several common bacterial host strains, it is especially suitable for the protease-defective expression host BL21 strains. If lysis is inefficient for a particular bacterial strain, freeze cells before extraction.

6. Whole soluble proteins fraction prepared with this Protein Lysis buffer are compatible with Better BCA Protein Assays (SK3051). Inclusion bodies in denaturing agents are compatible with our Non-Interfering Protein Concentration Determination Kit (SK3071).