

SoluLyse™

Reagent in Tris Buffer

amsbio

| Cat. No. | Content | Quantity |
|----------|----------------------------------|----------|
| L200500 | SoluLyse™ Reagent in Tris Buffer | 500 ml |
| L200125 | SoluLyse™ Reagent in Tris Buffer | 125 ml |

| | |
|---------------------------|---|
| Composition | Proprietary detergent in 20 mM Tris•HCl, pH 7.5 |
| Shipping Condition | Room temperature. |
| Storage Condition | Room temperature. |

| RELATED PRODUCTS | Catalog # |
|--|-----------|
| SoluLyse™ Bacterial Protein Extraction Reagent, 500 ml | L100500 |
| SoluLyse™ Bacterial Protein Extraction Reagent, 125 ml | L100125 |
| TurboCells BL21(DE3) Chemically Competent E. coli | C302020 |
| TurboCells BL21(DE3)pLysS Chemically Competent E. coli | C303020 |
| Blazing Blue™ Protein Stain (0.5 liters) | MTDB01 |
| WesternPro™ Chromogenic Detection HRP (Rabbit) Kit | D602200 |

INTRODUCTION SoluLyse™ Reagent in Tris Buffer provides a highly efficient yet gentle method for extracting proteins from bacteria cells. This reagent utilizes a proprietary nonionic detergent in 20 mM Tris-HCl (pH 7.5). With the SoluLyse™ reagent, there is no need for time-consuming and cumbersome mechanical disruption methods such as sonication. In addition, the SoluLyse™ reagent offers several fold increase in the yield of soluble proteins when compared with other commercial lysis reagents. Treatment of bacterial cells with the SoluLyse™ reagent results in rapid and efficient extraction of proteins that are suitable for affinity purification and analysis. It was demonstrated to work with both the GST and 6xHis purification systems. Additional components such as DNase I, lysozyme, protease inhibitors, salts, chelating agents, etc., may be added to the reagent. The SoluLyse™ reagent may also be used for preparation of high purity inclusion bodies from total cell lysates.

MATERIALS AND METHODS

A) GENERAL CONSIDERATIONS

- I. **E. coli strain:** Protein extraction efficiency is strain dependent. The SoluLyse™ reagent is especially effective in extracting proteins from the commonly used BL21 strains. The use of pLysS or pLysE strains enhances extraction due to the activity of T7 lysozyme. However, lysates from these strains have higher viscosity. DNase I may be added to clarify the lysates when these strains are used.
- II. **Buffer compatibility:** Tris buffers are recommended for subsequent purification and other applications following protein extraction with the SoluLyse™ reagent. The SoluLyse™ reagent is compatible with EDTA. However, the addition of EDTA will interfere with protein binding to metal-chelating resins used in purification of 6xHis-tagged proteins. For some proteins, better extraction and purification results are achieved with SoluLyse™ Reagent in Phosphate Buffer (Cat. No. L200500 and L200150).
- III. **Use of lysozyme:** Although the SoluLyse™ reagent is compatible with lysozyme, lysozyme is not needed to achieve efficient soluble protein extraction. However, for inclusion body preparation, lysozyme should be used to effectively digest the cell debris so that inclusion bodies can be fully released.

B) PROTOCOLS

For Obtaining Proteins in the Soluble Fraction

1. Harvest cells from 1.5 ml bacterial culture (OD₆₀₀ 1.5 – 3.0) at 10,000 rpm for 10 minutes in a microcentrifuge.
NOTE: For larger volumes, e.g. 40-250 ml of bacterial culture, pellet cells by centrifugation at 3,500 g for 10 minutes.
2. Remove all media by aspiration.
NOTE: The cells can either be used fresh or frozen at –80°C. Protein extraction is typically more effective with frozen cells.
3. Resuspend the pellet in 150 µl of SoluLyse™ Reagent by gently pipetting up and down until the cell suspension is homogenous. Rotate the tube for an additional 10 minutes at room temperature. Do not vortex.
NOTE: If the pellet was harvested from 40 ml of culture, resuspend in 3 ml of SoluLyse Reagent. If the pellet was from 250 ml of culture, 15-20 ml of SoluLyse Reagent should be used. If BL21(DE3)pLysS strain is used, add 1 KU/ml of DNase I at this step.
4. Centrifuge at 14,000 rpm for 5 minutes to separate the soluble and insoluble fractions. The soluble protein is in the supernatant.
NOTE: For larger volumes, separation of soluble and insoluble fractions can be achieved by centrifugation at 27,000 g for 15 minutes.
5. Transfer the supernatant to a clean tube and resuspend the insoluble fraction in 300 µl SoluLyse™ Reagent. Use 10 µl 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 2X volume of SoluLyse™ Reagent used in Step 3.

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For Inclusion Body Purification

6. Follow steps 1 through 4 above for soluble protein extraction.
7. Resuspend the pellet from step 4 in the same volume of SoluLyse™ Reagent used in step 3 above.
8. Add lysozyme to the resuspended pellet to a final concentration of 400 µg/ml. Vortex for 1 minute and incubate at room temperature for 5 minutes.
9. Add 6X volume of 1:20 diluted SoluLyse™ Reagent to the suspension and vortex for 1 minute.
10. 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.
 Step 4 and 5 may be repeated for multiple rounds of purification if necessary.
11. Resuspend the pellet of the purified inclusion bodies in the buffer of your choice.

LICENSE

The purchase price paid for the SoluLyse™ Bacterial Protein Extraction Reagent grants end users a non-transferable, non-exclusive license to use the kit and/or its components for **internal research use only** as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis. Separate licenses are available for non-research use or applications. **The SoluLyse™ Bacterial Protein Extraction Reagent is not to be used for human diagnostic or included/used in any drug intended for human use.** Care and attention should be exercised in handling the product by following appropriate research lab practices.

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