Bacterial Protein Extraction Reagent

L1507331Z

Storage 2-8℃.

Shipping Shipped under low temperature conditions. Please store under the specified storage conditions immediately upon receipt.

Introduction

Bacterial Protein Extraction Reagent, also named Active Bacterial Protein Extraction Reagent, is a lysis buffer designed for isolation of native active bacterial proteins. It supports direct cell lysis and protein extraction with rapid processing speed, superior quality and high yield, eliminating the need for sonication or high-pressure homogenization. Optimized for endogenous soluble proteins of Escherichia coli and recombinant proteins expressed in E. coli, this reagent is perfectly suited for high-throughput rapid screening of protein expression.

This reagent works well with freshly harvested bacterial cells and frozen cell pellets, and delivers particularly excellent extraction efficiency for frozen samples. The required reagent volume is calculated based on the weight of cell pellets. EDTA-free protease inhibitor cocktails, salts and reducing agents can be directly supplemented into the reagent system. Efficient protein extraction can be accomplished without adding exogenous lytic enzymes. The reagent recovers soluble proteins and removes debris and contaminants attached to the surface of inclusion bodies to prepare high-purity inclusion body samples. Note that it cannot solubilize inclusion bodies; it only serves as a washing reagent to purify intact inclusion bodies. It is applicable to a broad range of biochemical and molecular biology applications, and compatible with routine downstream assays including protein purification, ELISA and Western blot. For protein purification: Ni-TED resins are recommended for His-tagged protein purification. If Ni-IDA, Ni-NTA, GST-tagged or MBP-tagged proteins are to be purified, the lysate is advised to be diluted 10-fold or desalted by dialysis before purification.

Protocols

1 Mini-scale Soluble Protein Extraction

Mini-scale extraction is used to verify target protein expression and expression level prior to preparative large-scale extraction.

1.1 Culture recombinant strains and induce target protein expression following standard culture protocols.

1.2 Take 1.5 mL bacterial suspension with OD_{600} ranging from 0.5 to 2.0, centrifuge at 12,000–16,000 g for 2 min at 4 °C or ambient temperature, then discard supernatant.

1.3 Resuspend cell pellet in 0.2–0.4 mL bacterial protein extraction reagent; briefly vortex to achieve complete resuspension, followed by incubation at room temperature for no less than 1 min.

1.4 Centrifuge at 12,000–16,000 g (16,000 g preferred) for 5 min at 4 °C.

1.5 Carefully aspirate supernatant containing soluble protein. Avoid touching the pellet during supernatant collection to prevent contamination with excessive non-specific impurities.

1.6 Determine the localization of target protein (soluble supernatant vs insoluble pellet) via SDS-PAGE or Western blot analysis. Recommended loading volume per sample for SDS-PAGE: 5–15 μL.

2 Large-scale Soluble Protein Extraction

2.1 Culture recombinant strains and induce target protein expression per standard procedures.

2.2 Harvest 250 mL bacterial culture at OD_{600} ≈ 2.0, centrifuge at 5,000 g for 10 min at 4 °C or ambient temperature; approximately 1 g wet cell pellet is obtained after supernatant removal. The pellet can be lysed immediately or frozen for later lysis. Freeze-thaw treatment generally improves total protein yield but may impair the bioactivity of certain sensitive proteins.

2.3 Resuspend pellet with 20–50 mL Bacterial Protein Extraction Reagent per gram of wet cells (supplement with appropriate protease inhibitors before use), and homogenize by repeated pipetting. Less reagent yields higher protein concentration with compromised extraction efficiency; excess reagent improves lysis efficiency while lowering final protein concentration. Minimal reagent volume is recommended when abundant wet cell pellets are available. For optimal results, supplement with nuclease or recombinant universal nuclease to a final concentration of 50 U/mL to reduce lysate viscosity.

2.4 Incubate at room temperature for no less than 1 minute; the incubation time can be extended appropriately to achieve complete protein extraction.

2.5 Centrifuge at 12,000–16,000 g (16,000 g preferred) for 10 min at 4 °C.

2.6 Gently collect supernatant with soluble protein; contact with cell pellet during aspiration introduces high levels of miscellaneous proteins.

2.7 Identify target protein distribution in supernatant or insoluble pellet by SDS-PAGE or Western blot; recommended sample loading volume: 5–15 μL per well for SDS-PAGE.

Troubleshooting

1. Low yield of target protein

(1) Incomplete bacterial lysis. Repeated freeze-thaw cycles or supplementation with lysozyme facilitates cell rupture and improves protein recovery.

(2) Excessively high sample viscosity. Addition of nuclease or recombinant universal nuclease reduces viscosity and enhances soluble protein extraction.

(3) Target protein degradation. Supplement protease inhibitors to mitigate proteolysis.

(4) Poor intrinsic expression level of target protein. Increase IPTG dosage, prolong induction duration or optimize induction temperature; alternatively verify plasmid construction or switch to alternative expression strains.

(5) Target protein is insoluble. Inspect the post-centrifugation pellet to confirm inclusion body formation.
(6) Insufficient volume of extraction reagent. Appropriately increase the dosage of lysis reagent.
2. Turbid soluble protein lysate
(1) Under-dosed extraction reagent. Supplement additional extraction buffer accordingly.
(2) Inadequate bacterial lysis. Supplement lysozyme during extraction, which commonly clarifies the final protein solution.
(3) Overlong frozen storage of purified protein lysate. It is recommended to complete downstream assays within 1–2 weeks after protein extraction.
(4) Insufficient centrifugal force or insufficient spinning duration for supernatant-pellet separation. Centrifuge at 12,000–16,000 g (16,000 g recommended) for 10 min at 4 °C, or extend centrifugation time / elevate relative centrifugal force.
(5) Aggregation of expressed target protein. Add glycerol to a final concentration of 40–50% to inhibit protein aggregation and precipitation; alternatively isolate target protein via ammonium sulfate precipitation followed by resolubilization.
(6) Reduced protein solubility at low temperature. Warm the sample to ambient temperature to improve solubility and clarify turbidity; increasing reagent dosage is another feasible remedy.
3. Highly viscous soluble protein lysate
(1) Insufficient addition of extraction reagent. Increase the usage of extraction reagent properly.
(2) Supplement nuclease or recombinant universal nuclease to decrease sample viscosity.

Precautions

1. Protease inhibitor cocktail (Cat. No. P776695, P665818) or 100 mM PMSF (Cat. No. P301906) is recommended for addition to this product to inhibit protein degradation.
2. Recombinant universal nuclease (Cat. No. O775185, rp221814) or standard nuclease (Cat. No. B401536) is recommended to reduce the viscosity of protein lysates.
3. This product adopts a Tris-HCl buffer system, and the extracted protein samples are compatible with purification procedures using the same buffer system.
4. For difficult-to-lyse strains with unsatisfactory extraction performance, freeze-thaw treatment is recommended, which generally further improves protein extraction efficiency.
5. If samples extracted with this reagent will be purified via metal chelation chromatography, EDTA-containing reagents should generally be avoided. However, Aladdin His-tag Purification Resin (reduction-resistant chelating type) is compatible with EDTA, so lysates containing EDTA can still be used for purification of His-tagged proteins.
6. This product is for scientific research use by professionals only. It shall not be used for clinical diagnosis, medical treatment, food or pharmaceutical production, nor stored in residential areas. Wear a lab coat and disposable gloves during operation for personal safety and health.