UltraBio™ Bacterial Active Protein Extraction Reagent

B751647

Store at room temperature for up to 2 years, or store at 4℃ or -20℃ for long storage.

Introduction:

UltraBio™ Bacterial Active Protein Extraction Reagent allows for rapid extraction of proteins in E. *coli* without the need of ultrasonication or high-pressure crushing. The extracted proteins are of high quality, high yield, and high activity. It can be used not only to extract soluble proteins, but also to remove cell debris adhering to the surface of inclusion bodies to obtain high-purity inclusion bodies. It should be noted that this product cannot be used to extract proteins from inclusion bodies. This product is optimized to be suitable for BL21 bacteria,DH5α, JM109, and other similar bacteria as well. This product is a buffer solution containing 40mM Tris (pH 8.0) and a specific mild non-denaturing detergent that has been carefully selected to ensure that proteins can be extracted efficiently with activities well preserved. The extracted proteins can maintain their original spatial structures and biological activities, and can be used in a variety of biochemistry and cell biology applications, such as purification of His- or GST-tagged proteins, ELISA, Western Blot, IP, enzyme activity assay, and fluorescent protein assay, etc. This product can quickly and efficiently lyse bacteria, extracting cytosol and nuclear protein through a one-step method (15min) without ultrasonication or high-pressure crushing. Compared with other methods such as ultrasonication, high-pressure crushing and lysozyme treatment, target protein obtained using this product usually have higher yield and activity. The protein concentration of sample lysate obtained using this product can be determined with Aladdin's BCA Protein Assay Kit or the Detergent Compatible Bradford Protein Assay Kit.

Notes:

We recommend adding Protease inhibitor cocktail for bacterial cell extracts (100X), Protease inhibitor cocktail for purification of His-tagged proteins (100X) or PMSF (100mM) to this product to inhibit protein degradation. We also recommend adding EnzymoPure™ Super Nuclease to this product to reduce the viscosity of the extracted protein.

This product is formulated in Tris-HCl buffer. Same buffer system can be used for protein purification after protein extraction.

If the extraction effect is not satisfactory for some special strains, freezing and thawing of bacteria can be attempted, which can usually further improve the protein extraction.

If the extracted His-tag recombinant protein is going to be purified with nickel column, EDTA should not be used generally for protein extraction, as EDTA will destroy most metal chelation purification resins including the Ni-NTA resin. However, His-tag Purification Resin that is EDTA

compatible can be used to purify His-tagged proteins from protein extracts containing EDTA.
This product is for R&D only. Not for drug, household, or other uses.
For your safety and health, please wear a lab coat and disposable gloves during the operation.

Instructions for Use:

1. Small-scale extraction of soluble proteins

This extraction method is usually used to determine whether the target protein is expressed.
a. Follow conventional methods to cultivate recombinant protein expression strains and induce the expression of target proteins.
b. Take 1.5ml of bacterial culture with an OD600 of 0.5-2.0, centrifuge at 12,000-16,000×g at 4℃ or room temperature for 2min, and discard the supernatant.
c. Resuspend bacteria with 0.2-0.4ml of UltraBio™ Bacterial Active Protein Extraction Reagent by pipetting or vortex. Incubate at room temperature for 15 minutes.
d. Centrifuge at 12,000-16,000×g (better at 16,000×g) at 4℃ for 5min.
e. Carefully aspirate the supernatant containing soluble proteins into a new centrifuge tube. Do not touch the pellet when aspirating the supernatant.
f. Check the target protein level in the supernatant by SDS-PAGE or Western blot. The recommended loading volume of each sample for SDS-PAGE is 5-15μl.

2. Large-scale extraction of soluble proteins.

a. Follow conventional methods to cultivate recombinant protein expression strains and induce expression of target proteins.
b. Collect 250ml of bacterial culture with an OD600 of about 2.0, centrifuge at 5,000×g at 4℃ or at room temperature for 10 minutes, and discard the supernatant to obtain about 1g of bacteria pellet which can be lysed immediately or frozen at -20℃ to be lysed later. Higher protein yield can be obtained from frozen bacteria, but the freeze-thaw may have a negative effect on the activity of some proteins.
c. Add 20-50ml of UltraBio™ Bacterial Active Protein Extraction Reagent (appropriate protease inhibitor can be added before use) per gram of bacteria pellet, and resuspend bacteria completely by pipetting. More or less Protein Extraction Reagent can be used to reduce or increase the protein concentration in the crude extract. If it is easier to obtain more wet bacteria, it is recommended to use less extraction reagents. Additionally, add lysozyme and EDTA at a final concentration of 2mg/ml and 2mM, respectively, to further improve the protein extraction. EnzymoPure™ Super Nuclease can also be added to reduce the viscosity of the crude extract.
d. Incubate at room temperature for 15 minutes.
e. Centrifuge at 12,000-16,000×g (better at 16,000×g) for 10 minutes at 4℃ or room temperature.
f. Carefully aspirate the supernatant containing soluble proteins into a new centrifuge tube. Do not touch the pellet when aspirating the supernatant.
g. Check the target protein level in the supernatant by SDS-PAGE or Western blot. The recommended loading volume of each sample for SDS-PAGE is 5-15μl.

FAQ:

1. The yield of target protein is low.

a. Insufficient bacterial lysis. Repeated freeze-thaw of bacteria or the addition of lysozyme can improve the protein extraction.

b. The viscosity of the sample is too high. The addition of EnzymoPure™ Super Nuclease or DNase I can reduce the viscosity of the crude extract and facilitate the extraction of soluble proteins.

c. Target protein is degraded. Add protease inhibitors in Protein Extraction Reagent can reduce the degradation of target proteins. We recommend using Protease Inhibitor Cocktail for Bacterial Cell Extracts (100X), Protease Inhibitor Cocktail for Purification of His-tagged Proteins (100X) or PMSF (100mM).

d. The expression level of target protein is low. We recommend adding a higher concentration of IPTG, extending the induction time, adjusting the induction temperature, verifying the expression plasmid or using other protein expression strains.

e. Target protein may be insoluble. Check the precipitate after centrifugation to determine whether the target protein is in inclusion bodies.

f. The amount of Protein Extraction Reagent added is insufficient. Increase the amount of Protein Extraction Reagent appropriately.

2. The obtained soluble protein solution is turbid and opaque.

a. The amount of Protein Extraction Reagent added is insufficient. Appropriately increase the amount of extraction reagents.

b. Insufficient bacterial lysis. Adding lysozyme during extraction is likely to make the final protein solution clear.

c. The obtained protein solution has been frozen for too long. The protein solution should be used within 1-2 weeks.

d. The centrifugal force or centrifugal time when separating the protein supernatant from the precipitate is not enough. Make sure to centrifuge at 14,000×g for 15 minutes, use a greater centrifugal force or centrifuge for a longer time.

e. The expressed target protein aggregates. Adding glycerol to a final concentration of 40-50% can usually prevent protein aggregation and precipitation, or use ammonium sulfate precipitation to precipitate the target protein from the extracts and try to dissolve it.

f. Low temperature leads to a decrease in protein solubility. Equilibrate to room temperature may increase the solubility of the protein and make the solution clear. Increasing the amount of Protein Extraction Reagent can also solve the problem.

3. The obtained soluble protein solution is viscous.

a. The amount of Protein Extraction Reagent used for protein extraction is insufficient. Appropriately increase the amount of Extraction Reagent.

b. Add appropriate amount of EnzymoPure™ Super Nuclease or DNase I to reduce the viscosity.