Active Protein Extraction Reagent (Bacteria)

Introduction

Active Protein Extraction Reagent (Bacteria) is a gentle, easy-to-use cell lysis reagent consisting of a native detergent containing phosphate buffer, pH 8.0, which effectively destroys cells and dissolves native or recombinant proteins without denaturation. This product is mainly used for direct lysis and extraction of soluble bacterial active proteins expressed by E. coli itself and the expressed recombinant proteins; At the same time, although it cannot be used to solubilize inclusion bodies, it can be used to wash cell debris that removes adhesion from the surface of inclusion bodies to obtain high-purity inclusion body proteins. This product is optimized for use with BL21, DH5α, JM109 and other similar bacteria.

The proteins extracted from this product generally maintain the original structure and biological activity of the protein, and can be used for a variety of biochemical and molecular biology purposes, while being compatible with downstream applications, including affinity chromatography (e.g., fixed metal affinity chromatography, glutathione chromatography), SDS-PAGE and protein quantification (e.g., BCA protein assay), IP, enzyme activity assays, fluorescent protein assays, and more. Depending on the application, protease inhibitors, salts, reducing agents, denaturants, and chelating agents can be added to the reagent.

This product is a special lysis reagent for one-step rapid (15 min) lysis and extraction of bacterial cytoplasmic proteins and intranuclear proteins of nucleated cells. At the same time, compared with common cell disruption methods, such as ultrasonic disruption, high-pressure sterilization, and lysozyme treatment, this product can usually obtain higher yields and higher activity of soluble protein of interest. Products are compatible with Sigma's CelLytic™ series as well as Thermo's B-PERThe TM series is very similar, and the use effect and use are very similar, and in many cases, they can be considered to replace each other.

Protocol

1. Mini-extraction of soluble proteins (also used in small-scale experiments before large-scale extraction)

    a. Take 1.5 mL of bacterial solution with an OD600 of 0.5-2.0, centrifuge at 12,000-16,000 g at 4°C or room temperature for 2 min, and then discard the supernatant.

    b. Resuspend the cells with 0.2 - 0.4 mL of Active Protein Extraction Reagent (Bacteria). (You can briefly vortex the mixture to ensure full resuspension.) Then incubate at room temperature for 15 minutes.

    c. Centrifuge at 12,000 - 16,000 g (16,000 g is recommended) at 4 °C for 5 minutes.

d. Carefully aspirate the supernatant, which is the soluble protein in the bacteria that needs to be extracted. In this step, it is necessary to pay attention not to touch the precipitate when aspirating the supernatant, otherwise the extracted product will contain more insoluble heteroproteins.

e. Detection: The protein of interest in the supernatant can be detected by SDS-PAGE or Western blot. If the extracted protein is insoluble, it may be present in the precipitate. The recommended sample loading for SDS-PAGE electrophoresis is 5-15 μL.

2. Extraction of large quantities of soluble protein.

a. 250 mL of OD600 was about 2.0, centrifuged at 5,000 g at 4°C or room temperature for 10 min, and then the supernatant was discarded. This step typically yields approximately 1 g of wet bacteria, which can be cryopreserved and then lysed for higher protein yields, but freeze-thaw may have a negative impact on the activity of some proteins, and direct non-freeze-thaw lysis may be considered.

b. Operate according to the proportion of adding 20 - 50 mL of Active Protein Extraction Reagent (Bacteria) for every 1 g of wet bacteria. During the process, use a pipette to repeatedly blow and mix evenly. Subsequently, incubate at room temperature for 15 minutes.

***Note:**

1. Before adding Active Protein Extraction Reagent (Bacteria), it is advisable to consider adding an appropriate amount of protease inhibitors to protect the extracted proteins, such as Protease Inhibitor Cocktail for bacterial cell extracts (100 X in DMSO) (Cat. #: K1024).
2. The amount of Active Protein Extraction Reagent (Bacteria) can be added according to the experimental requirements. If the protein extraction efficiency is pursued, the amount of reagent can be increased, and the concentration after protein extraction can be reduced. When the number of wet bacteria is large and easy to obtain, it is recommended to reduce the addition of reagents on the basis of satisfying the extraction amount.
3. For best results, consider adding lysozyme at a final concentration of 2 mg/mL and EDTA at 2 mM to further improve extraction, and nuclease at a final concentration of 50 U/mL to degrade nucleic acids to reduce viscosity.

c. 12,000-16,000 g (16,000 g recommended) Centrifuge at 4°C or room temperature for 10 min.

d. Carefully aspirate the supernatant, which is the soluble protein in the bacteria that needs to be extracted. In this step, it is necessary to pay attention not to touch the precipitate when aspirating the supernatant, otherwise the extracted product will contain more insoluble heteroproteins.

e. Detection: The protein of interest in the supernatant can be detected by SDS-PAGE or Western blot. If the extracted protein is insoluble, it may be present in the precipitate. The recommended sample loading for SDS-PAGE electrophoresis is 5-15 μL.

FAQs

Issue	Cause	Suggestions
Low yield of target protein	Insufficient lysis	Repeated freeze-thaw bacteria promote cell rupture or the addition of lysozyme
	The viscosity is too high	The addition of nucleases such as DNase I reduces the viscosity of the sample
	Degradation of the protein of interest	The addition of the protease inhibitor Cocktail reduces the degradation of the protein of interest
	The expression level of the protein of interest is low	You can choose to add a higher concentration of IPTG, extend the induction time, adjust the induction temperature, etc., and consider checking the constructed plasmid, or choose other protein-expressing strains
	The protein of interest may be insoluble	Check the pellet after centrifugation to determine if the protein of interest has formed inclusion bodies
The final solution obtained is viscous	Too little protein extraction reagent is added	Increase the amount of protein extraction reagent appropriately
	The amount of protein extraction reagent added is insufficient	Increase the amount of extraction reagent appropriately
	Caused by macromolecular nucleic acids	The addition of nucleases such as DNase I reduces the viscosity of the sample
	The amount of protein extraction reagent added is insufficient	Increase the amount of extraction reagent appropriately
The solution is cloudy and opaque	Insufficient cell lysis	Add lysozyme appropriately
	Insufficient centrifugal force or centrifugation time for separating protein supernatants and pellets	Make sure to centrifuge at 14,000 g for 15 min, or use a higher centrifugal force or centrifuge longer
	Aggregation occurs with the expressed protein of interest	The addition of glycerol to a final concentration of 40-50% usually prevents protein aggregation and precipitation, or ammonium sulfate precipitation can be used to precipitate the protein of interest from the extraction reagent and then try to dissolve it
	Low temperatures lead to a decrease in protein solubility	Returning to room temperature and increasing protein solubility may make the solution clear. In this case, increasing the amount of extraction reagent can also solve this problem

Note

1. For special strains, if the extraction effect is not ideal, freeze-thaw treatment can be considered.
2. Storage and transportation: 2 years at 4°C; Blue Ice Transport.
3. Related product recommendations

Catalog No.	Product name	Description
K1024	Protease Inhibitor Cocktail for bacterial cell extracts (100 X in DMSO)	Inhibits protein degradation during bacterial lysis and protein extraction
K1015	Phosphatase Inhibitor Cocktail (2 Tubes, 100X)	Full version of protease and phosphatase inhibitors (2 tubes)
K4004	Phosphatase Inhibitor Cocktail (1 Tube, EDTA-Free, 100X in ddH_2O)	Full version of phosphatase inhibitor (1 tube)
K4005	Protease and Phosphatase Inhibitor Cocktail (EDTA plus, 100X in ddH_2O)	Protease and phosphatase inhibitors (2 tubes)
K4006	Protease and Phosphatase Inhibitor Cocktail (EDTA Free, 100X in ddH_2O)	Protease and phosphatase inhibitors (1 tube, EDTA-free)
K1017	Deacetylase Inhibitor Cocktail (100× in 70% DMSO)	Maintain the acetylation state of proteins
K1088	DNase I (RNase-free)	Endonucleases for single- or double-stranded DNA
K1088R	DNase I (GMP-grade)	Endonuclease for single - stranded or double - stranded DNA (GMP-grade)
K4101	BCA Protein Assay Kit	Protein Concentration Assay Kit, BCA method
K4102	Micro BCA Protein Assay Kit	Classic BCA protein concentration determination kit, suitable for low protein concentration samples (0.5~20 μg/mL)
K4104	Detergent Compatible Bradford Protein Assay Kit	Classic Bradford Protein Concentration Assay Kit (Detergent Compatible)
K4105	Bovine Serum Albumin Standard (5 mg/mL)	Protein standard BSA

4. This product is for scientific research use only.

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