Active Protein Extraction Reagent (Mammal)

Introduction

Active Protein Extraction Reagent (Mammal) is a gentle, easy-to-use cell lysis reagent consisting primarily of a native detergent (Tris, pH 8.0). This product is mainly used for the extraction of active proteins from cytoplasmic proteins, nuclear proteins and membrane proteins of mammalian cells or tissues. If you wish to remove the detergent from the protein solution extracted using this product, you can do so by dialysis or ultrafiltration.

The proteins extracted by this product can usually maintain their original structures and biological activities well and can be used for a variety of biochemical and molecular biological purposes. Meanwhile, they are compatible with downstream applications, including the separation and purification of tagged proteins, such as His, GST, HA, Flag, Myc, V5 and other tagged proteins; conventional biochemical analyses, such as Western blot, IP/Co-IP, ELISA, etc.; the detection of reporter gene enzyme activities, such as luciferase, β-gal, alkaline phosphatase, CAT, etc.; and the detection of protein kinase activities, such as PKA, PKC and tyrosine kinase, etc.

This product is very similar to Thermo's M-PERTM series and Sigma's CelLytic™ series. Their application effects and uses are also quite close, and they can often be substituted for each other as options.

Protocol

1. Preparation of protein extraction solution

   Take an appropriate amount of Active Protein Extraction Reagent (Mammal) and place it in an ice bath for later use. Refer to the following sample types for specific amounts.

***Note:** Inhibitor Cocktail (such as Protease Inhibitor Cocktail K4001 for mass spectrometry, Protease Inhibitor Cocktail K1007/K4002, Phosphatase Inhibitor Cocktail K1015/K4004) and nucleases (such as DNase I K1088) can be added according to the experimental requirements.

2. Protein extraction of suspension cells

   a. Collect cells: Centrifuge the cells at 2,500 g at 4 °C or room temperature for 10 minutes and discard the supernatant.

   b. Wash cells (optional): Wash cells in PBS, centrifuge at 2,500 g at 4 °C or room temperature for 10 min, discard the supernatant.

   c. Lyse cells: Tap the bottom of the centrifuge tube to properly disperse the cell pellet, then add the

prepared pre-chilled protein extraction solution (step 1) and mix well by pipette pipette, and finally vortex at medium speed for about 5 seconds and then incubate on ice for 10 minutes (add amount according to Table 1).

***Note:** For routine assays, no additional incubation is required after the addition of extraction reagents, but additional incubation time is required to increase yield if more protein is desired, or for some proteins that are more difficult to lyse extracted. Additional incubation time may increase protein yields ranging from 10 to 50%, which will vary from cell to cell.

d. Centrifuge at 14,000 g for 5 - 10 minutes at 4°C.

e. Transfer the supernatant to a new centrifuge tube, i.e., the insect cells obtained have soluble proteins. If necessary, consider retaining the precipitate for subsequent analysis.

f. Assay (optional): Immediately used for subsequent analytical assays, such as WB, etc.

g. Storage: Store at -20°C or -80°C for later use.

3. Protein extraction of adherent cultured insect cells.

a. Wash cells (optional): Aspirate the culture medium and add an appropriate amount of PBS, shake gently, and then aspirate the PBS.

***Note:** Washing or not can be selected on a case-by-case basis. If the culture medium and the drug added to the culture medium will not affect the subsequent experiments, the cell washing can be omitted; If the impact is large, you need to repeat the washing again.

b. Lyse cells: Aspirate the cell culture or wash solution, add pre-chilled protein extraction solution (step 1 preparation, refer to Table 1 for dosage), and incubate on ice for 2-10 min. During the ice incubation, the culture vessel can be placed on a horizontal or side-swinging rocker with vigorous shaking, tapping the outer wall of the cell culture flask on the cell culture side, or scraping the adherent cells with cells. Usually after 5-6 minutes of direct incubation, the insect cells will gradually detach from the surface of the culture vessel.

***Note:** For routine assays, no additional incubation is required after the addition of extraction reagents, but additional incubation time is required to increase yield if more protein is desired, or for some proteins that are more difficult to lyse extracted. Additional incubation time may increase protein yields ranging from 10 to 50%, which will vary from cell to cell.

c. Suspend the cell extracts by pipette and transfer to a centrifuge tube at 14,000 g at 4 °C for 5-10 min.

d. Transfer the supernatant to a new centrifuge tube, i.e., the insect cells obtained have soluble proteins. If necessary, consider retaining the precipitate for subsequent analysis.

e. Assay (optional): Immediately used for subsequent analytical assays, such as WB, etc.

f. Storage: Store at -20°C or -80°C for later use.

4. Tissue protein extraction

a. Tissue pretreatment: Tissue is cut into fine pieces or frozen with liquid nitrogen and ground into a powder.

b. Homogenization and extraction: Add the protein extraction solution (from Step 1) at a ratio of 200 - 400 μL of extraction reagent per 20 mg of tissue. Then homogenize the mixture using a glass homogenizer.

c. Incubate on ice for 2-10 min. Depending on the tissue type and the purpose of the experiment, the tissue can be fully lysed by moving on to the next steps or by continuing to incubate on ice for 2–20 minutes (or in a shaker for further improvement if necessary).

***Note:** For routine assays, no additional incubation is required after the addition of extraction reagents, but additional incubation time is required to increase yield if more protein is desired, or for some proteins that are more difficult to lyse extracted. Additional incubation time may increase protein yields ranging from 10 to 50%, which will vary from cell to cell.

d. Centrifuge at 14,000 g for 5 - 10 minutes at 4°C.

e. Transfer the supernatant to a new centrifuge tube, i.e., the insect cells obtained have soluble proteins. If necessary, consider retaining the precipitate for subsequent analysis.

f. Assay (optional): Immediately used for subsequent analytical assays, such as WB, etc.

g. Storage: Store at -20°C or -80°C for later use.

Table 1 Dosage Table of Active Protein Extraction Reagent (Insect) in Cell Culture Vessels of Different Specifications

Multiple Well Plates or Dishes	Growth area (cm^2)	Volume of Extraction Reagent (μL)
10-cm dish	55	500-1000
6-cm dish	21	200-400
6-well plate	9.5	100-200
12-well plate	3.8	50-100
24-well plate	1.9	25-50
48-well plate	0.95	12.5-25
96-well plate	0.32	5-10

FAQs

Issue	Cause	Suggestions
Low yield of soluble protein	Inadequate cell or tissue lysis	Extend the incubation time on ice or shake appropriately during the incubation, or increase the amount of protein extraction reagent used appropriately
	Protein degradation occurs	The addition of protease inhibitors inhibits protein degradation
	Tissue homogenization is less sufficient	Extend the homogenization or grinding time appropriately
	The consistency of the protein solution is too high	Increase the amount of extraction reagent, or nuclease, to reduce the viscosity
The amount of exogenously expressed protein is low or undetectable	Low expression of exogenous proteins	Consider optimizing your protocol for cell plasmid transfection or viral infection
	The plasmid or virus is incorrect	Consider setting up an appropriate positive control and sequencing and checking the plasmid or virus-packed plasmid, and testing and

		validating expression.

Notes

1. All steps of extracting protein samples should be performed on ice or at 4 °C to ensure protein activity.
2. Appropriate protease inhibitors and phosphatase inhibitors can be added to the extraction reagent as needed.
3. If the extracted His-tagged recombinant protein is subsequently used for purification of nickel columns, etc., the addition of EDTA-containing reagents should generally be avoided.
4. Storage and transportation: 2 years at 4°C; Blue Ice Transport.
5. Related product recommendations

Catalog No.	Product name	Description
K1007	Protease Inhibitor Cocktail (EDTA-Free,100X in DMSO)	Equivalent to Sigma P8340, it is used for cell and tissue extracts to increase protein stability.
K4001	Protease Inhibitor Cocktail (MS-SAFE, 50X in DMSO)	Mass Spectrometry (MS) - Compatible Protease Inhibitor Cocktail
K4002	Protease Inhibitor Cocktail (EDTA-Free,100X in DMSO)	Equivalent to Sigma S8830, it is used for cell and tissue extracts to increase protein stability.
K4003	Protease Inhibitor Cocktail (100X H_2O, EDTA Plus)	Equivalent to Sigma S8820, it is used for cell and tissue extracts to increase protein stability.
A2587	PMSF	Irreversible inhibitor of serine protease
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 ddH2O)	Full version of phosphatase inhibitor (1 tube)
K4005	Protease and Phosphatase Inhibitor Cocktail (EDTA plus, 100X in ddH2O)	Protease and phosphatase inhibitors (2 tubes)
K4006	Protease and Phosphatase Inhibitor Cocktail (EDTA Free, 100X in ddH2O)	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)	Endonucleases for single- 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

6. This product is for scientific research use only.

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