Active Protein Extraction Reagent (Insect)

Introduction

Active Protein Extraction Reagent (Insect) 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 insect active proteins from cytoplasmic proteins, nuclear proteins and membrane proteins of insect cells (such as Sf 9 and Sf 21) or insect tissues.

The extraction effect of this product for cytoplasmic proteins is better than that of nucleus and cell membrane proteins, and although it also has a good extraction effect on membrane proteins, the extraction efficiency of some insoluble proteins is relatively low. 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 from this product typically maintain their original structure and biological activity very well. They can be used for various biochemical and molecular biology applications, and are compatible with downstream applications, including the separation and purification of tagged proteins such as His, GST, HA, Flag, Myc, and V5. They are also suitable for routine biochemical analyses such as Western blotting, immunoprecipitation (IP)/co-immunoprecipitation (Co-IP), and ELISA. Additionally, they can be used for reporter gene enzyme activity assays, including luciferase, β-galactosidase, alkaline phosphatase, and CAT. Furthermore, they are applicable for detecting protein kinase activity, such as PKA, PKC, and tyrosine kinase.。

This product is compatible with Thermo'sI-The PERTM series is very similar, and the use effect and use are very similar, and many times you can choose to replace each other.

Protocol

1. Preparation of protein extraction solution

   Add 1 mL of ice-cold Active Protein Extraction Reagent (Insect) to every 0.5-2 × 10^7 insect suspension cells. For adherent insect cells, prepare the extraction reagent according to the parameters in Table 1 and place it on ice for later use.

***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 cultured insect cells.

a. Collect cells: Count the cells and determine the total number of cells. Centrifuge at 800 g at room temperature for 5 min, and discard the supernatant.

b. Wash cells (optional): Wash cells in PBS, centrifuge at 800 g for 5 min at room temperature, and discard the supernatant. If necessary, the wash can be repeated one more time.

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 with pipette pipette, and finally vortex at medium speed for about 5 seconds and incubate on ice for 10 minutes.

***Note:** If you want to get more protein, or for some proteins that are difficult to lyse extracted, additional incubation time will be required to improve yield.

d. Centrifuge at 12,000 - 16,000 g (16,000 g is recommended) for 15 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. If necessary, the wash can be repeated one more time. If the cells fall off during the washing process, the corresponding culture medium or PBS solution can be collected, centrifuged at 800 g for 5min, and the pellet can be collected, that is, the cells that have fallen off.

b. Lyse cells: Aspirate the cell culture medium or washing solution, add pre-chilled protein extraction solution (refer to Table 1 to add), and incubate on ice for 10 minutes. 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.

c. Pipette the cell extract to a centrifuge tube and centrifuge at 12,000-16,000 g (16,000 g is preferred) for 15 minutes at 4°C.

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.

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	The protein of interest does not easily solubilize, or forms inclusion bodies	The pellet was dissolved with SDS-PAGE loading buffer and stained with CBB or detected by WB
	The protein of interest is not expressed	Optimize expression conditions, check and check expression systems
	The protein of interest binds to the cell membrane	Try to dissolve the precipitate with a detergent that can better extract insect membrane proteins
Degradation of the protein of interest	The sample contained higher levels of protease	Add protease inhibitors to the extraction reagent
	The protein of interest is degraded at room temperature	All lysis and extraction steps are performed on ice or at 4 °C.
The cell extract protein concentration is too low or too high	Adjust the difference in the amount of extraction reagent used	According to the actual situation, the amount of extraction reagent should be adjusted appropriately.

Notes

1. All steps of extracting protein samples should be performed on ice or at 4°C to ensure protein activity.
2. 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.
3. Storage and transport: 12 months at 4°C; Blue Ice Transport.
4. 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

5. This product is for scientific research use only.

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