INSTRUCTIONS

I-PER® Insect Cell Protein Extraction Reagent

89802

1749.0

Number	Description
89802	**I-PER® Insect Cell Protein Extraction Reagent,** 250 ml, contains a proprietary non-ionic detergent in 130 mM NaCl, 25 mM Tris•HCl; pH 7.5 and a microbial growth inhibitor

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

I-PER® Insect Cell Protein Extraction Reagent extracts cytoplasmic protein from Sf9 and Sf21 insect cells grown in suspension and adherent cultures. The composition of I-PER® Reagent is compatible with downstream processing steps such as 6xHis-tagged protein purification and ion exchange chromatography. I-PER® Reagent is also compatible with Western blotting and the Pierce BCA Protein Assay.

Additional Materials Required

- Protease Inhibitor Cocktail (optional): Halt™ Protease Inhibitor Cocktail with EDTA (Product No. 78410). Avoid adding EDTA when extracting 6xHis-tagged proteins, as EDTA will inactivate most nickel-chelated chromatography resins used for purification.
- Phosphate Buffered Saline (PBS): 0.1 M sodium phosphate, 0.15 M sodium chloride; pH 7.2 (Product No. 28372)

Procedure for Protein Extraction from Suspension-cultured Insect Cells

Notes:

- Centrifugal forces and handling techniques detailed in the procedure must be followed exactly as indicated to avoid partial lysis of insect cells, which adversely affects protein yield.
- Approximately 0.4-1.5 mg/ml soluble protein can be obtained from 5×10^6 - 2×10^7 cells using 1 ml of the I-PER® Reagent. Adjust the amount of I-PER® Reagent used as needed.
- Perform all lysis steps at 4°C or on ice to decrease the rate of proteolysis.

A. Harvest the Insect cells

1. Use a hemacytometer to determine the number of cells per milliliter of culture.
2. Multiply the culture volume (ml) by the number of cells per milliliter to obtain the total number of cells.
3. Harvest cells by centrifugation at 800 × *g* for 5 minutes at room temperature.
4. Decant the growth media and save the cell pellet.

B. Wash the Insect cells

1. Add a volume of room temperature PBS to the pellet that is equal to the culture volume. Gently resuspend cells by pipetting.
2. Centrifuge cells at 800 × g for 5 minutes at ambient temperature and decant the supernatant.
3. Repeat Steps 1-2.

C. Lyse the Insect cells using I-PER® Reagent

Note: For best results, add protease inhibitors to the I-PER® Reagent immediately before use.

1. Add 1 ml of I-PER® Reagent per 5×10^6 - 2×10^7 cells to the washed cell pellet.
2. Resuspend cells by pipetting up and down. Vortex cells for 5 seconds at medium speed.
3. Incubate cells on ice for 10 minutes.
4. Centrifuge cells at 15,000 × *g* for 15 minutes at 4°C.
5. Carefully transfer the supernatant containing soluble proteins to a new tube. Avoid disrupting the pellet. Save the pellet, which contains insoluble protein and cellular debris, for further analysis.

Procedure for Protein Extraction from Monolayer-cultured Insect Cells

Important Note: Centrifugal forces and handling techniques detailed in the procedure must be followed exactly as indicated to avoid partial lysis of insect cells, which adversely affects protein yield.

A. Wash the Insect cells

1. Aspirate the media from the plate.
2. Gently add a volume of PBS to the plate that is equal to the culture volume. Be careful not to dislodge cells. Aspirate the PBS from the plate. Repeat this step.

   Note: To recover dislodged cells, centrifuge cells at 800 × *g*, wash the cell pellet with PBS and return cells to the plate.

B. Lyse the Insect cells using I-PER® Reagent

Note: For best results, add protease inhibitors to the I-PER® Reagent immediately before use.

1. Add an appropriate volume of I-PER® Reagent according to the following table:

Plate Size/Surface Area	Volume of I-PER® Reagent
100 mm dish	500-1,000 µl
60 mm dish	250-500 µl
6-well plate	200-400 µl per well
24-well plate	100-200 µl per well
96-well plate	50-100 µl per well
T-25 flask	500 µl per flask
T-75 flask	1.5 ml per flask

2. Incubate cells for 10 minutes at 4°C. Incubate plates on a shaker platform with vigorous shaking. Tap flasks on the side or use a cell scraper. Cells should appear detached after 5-6 minutes.
3. Use a pipette to transfer the cells and debris to a new tube. Tilt the plate or flask to collect all material.
4. Centrifuge tube at 15,000 × *g* for 15 minutes at 4°C.
5. Use a pipette to carefully transfer the supernatant containing soluble proteins to a new tube. Avoid disrupting the pellet. Save pellet containing insoluble protein and cellular debris for further analysis.

Additional Information

A. Purification of 6xHis-tagged Proteins

For optimal purification using immobilized nickel-chelated resin, adjust I-PER® Reagent cell extracts as follows:

- Adjust the salt concentration from 130 mM NaCl to the desired level (e.g., 300 mM NaCl) by directly adding an appropriate volume of 5 M NaCl.
- Add a volume of 1 M imidazole pH 7.5 for final concentration of 10-20 mM.

B. Please visit the web site for additional information relating to this product including the following:

- Tech Tip: Protein Stability and Storage
- Tech Tip: Convert Between Times Gravity (× *g*) and Centrifuge Rotor Speed (RPM)

Troubleshooting

Problem	Possible Cause	Solution
Protein of interest is not in soluble fraction	Protein of interest is insoluble	Solubilize the pellet in SDS-PAGE loading buffer and analyze by coomassie-stained gel or Western blot
	No expression of protein of interest	Optimize expression protocol
	Protein is associated with the cell membrane	Try to solubilize pellet with detergents known to extract membrane associated proteins
Cell extract is too dilute	Optimize amount of I-PER® Reagent	Use less I-PER® Reagent
Cell extract is too concentrated	Optimize amount of I-PER® Reagent	Use more I-PER® Reagent, or dilute cell extract with I-PER® Reagent
Protein of interest is degraded	Proteolysis	Use Halt™ Protease Inhibitor Cocktail and perform all lysis steps at 4°C

Related Thermo Scientific Products

78410	**Halt™ Protease Inhibitor Cocktail Kit**, sufficient to treat 200 ml of sample
78415	**Halt™ Protease Inhibitor Cocktail, EDTA-Free**, sufficient to treat 100 ml of sample
23225	**Pierce BCA Protein Assay Kit**, sufficient reagents to perform 500 standard tube assays or 5,000 microplate assays
78833	**NE-PER® Nuclear and Cytoplasmic Extraction Kit**, sufficient reagents for extracting 50 cell pellet fractions having packed cell volumes of 20 µl each
89826	**Mem-PER® Eukaryotic Membrane Protein Extraction Kit**, sufficient reagents for extracting 50 cell pellet fractions of 5×10^6 cells each
78501	**M-PER® Mammalian Protein Extraction Reagent,** 250 ml
25200-25244	**Precise™ Protein Gels** (see catalog or web site for a complete listing)
24612	**SilverSNAP® Stain Kit II**
24615	**Imperial™ Protein Stain,** 1 L

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.