PROMETHEUS™
Protein Biology Products

Instruction Manual

Insect Cell Protein Extraction Buffer

Catalog No.: 18-404 & 18-405

Introduction:
Our Insect Cell Protein Extraction Buffer has been designed to extract cytosolic proteins from SF9 and SF21 insect cells grown in suspension and adherent cultures. The supplied protein extraction buffer contains a proprietary nonionic detergent with salts and organic buffering agents (130 mM NaCl, 25 mM Tris-HCl, pH 7.5) and the extracted proteins are in their native form. This unique composition offers adaptability for different applications and compatibility with downstream applications such as 6x his-tagged protein purification, ion exchange purification, BCA Protein Assay, enzyme assays, ELISA, and Western Blot, etc.

Items Supplied:

Name	Cat. No. 18-404	Cat. No. 18-405	Storage Condition*
Insect Cell Protein Extraction Buffer	250 ml	500 ml	4^0C

**Shipped at ambient temperature and upon receiving, store at 4^0C.*

Additional Materials Needed (Not Supplied):
- Protease inhibitor Cocktail
- Phosphate Buffered Saline (PBS)

Important Note:
- Do not use EDTA as metalloprotease inhibitor, when extracting His-tagged proteins as it will inactivate most nickel-chelated chromatography resins, used for protein purification.
- All steps outlined in the protocol must be followed exactly as indicated to avoid partial lysis of insect cells, which adversely affects protein yield.
- Perform all lysis steps at 4^0C or on ice to minimize proteolysis.
- The volume of insect extraction buffer should be adjusted depending on the volume of insect cells used for a particular experimental condition. For example, 1ml insect protein extraction buffer should be used for each 2 x 10^7cells from insect cultures with approximate yield of 0.5 mg to 1.5 mg/ml soluble proteins.

Protocol for Extracting Protein from Suspension-cultured Insect Cells:

Harvest the Insect Cells
1. Use hemocytometer to calculate the number of insect cells per ml of cell culture. Multiply the culture volume (ml) by the number of cells per ml to obtain the total number of cells.
2. Pellet insect cells by centrifugation at 800 × g for 5 minutes at 4^0C.

Wash the Insect cells
3. Add a volume of room temperature PBS to the pellet equal to the culture volume. Gently resuspend cells by pipetting up and down.
4. Centrifuge cells at 800 x g for 5 minutes at ambient temperature and decant the supernatant.
5. Repeat Steps 3-4 once more.

Lyse the Insect cells

Note: *For best results, add protease inhibitors to the Insect Protein Extraction Buffer, before use.*

6. Add 1 ml of Insect Protein Extraction Buffer per $5x10^6$ – $2x10^7$ cells to the washed insect cell pellet.
7. Resuspend cells by pipetting up and down. Vortex cells for 5 seconds at medium speed.
8. Incubate cells on ice for 10 minutes.
9. Centrifuge cells at 15,000 x g for 15 minutes at 4^0C.
10. Carefully transfer the supernatant, which contains the soluble proteins to a new tube. ***NOTE:*** *Do not disrupt the pellet in the tube (centrifuged) and save it further analysis as it contains insoluble protein and cellular debris.*

Protocol for Extracting Proteins from Monolayer-cultured Insect Cells:

Wash the Insect cells

1. Aspirate the media from the culture plate.
2. Gently add a volume of PBS to the plate that is equal to the culture volume. Be careful not to dislodge the cells. Aspirate the PBS from the plate. Repeat this step once more.
   Note: *To recover dislodged cells, centrifuge cells at 800 x g, wash the cell pellet with PBS and return cells to the plate.*

Lyse the Insect cells

Note: *For best results, add protease inhibitors to the extraction buffer just before use.*

3. Add an appropriate volume of Insect according to the following table:

Plate Size/Surface Area	Volume of Insect Cell Protein Extraction Buffer
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-75 flask	1.5 ml per flask

4. Incubate cells for 10 minutes at 4^0C. 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.
5. Use a pipette to transfer the cells and debris to a new tube. Tilt the plate/ flask to collect all material.
6. Centrifuge the tube at 15,000 x *g* for 15 minutes at 4^0C.
7. Use a pipette to carefully transfer the supernatant containing the soluble proteins to a new tube.
   NOTE: *Do not disrupt the pellet in the tube (centrifuged) and save it further analysis as it contains insoluble protein and cellular debris.*

Additional Notes for Purification of 6xHis-tagged Proteins:

For optimal purification of His-tagged proteins, using immobilized nickel-chelated resin, adjust the EsayPrep Insect Protein Extraction Buffer as follows:

1. Increase the salt concentration from 130 mM NaCl (in the supplied buffer) to the desired level, e.g., 300 mM NaCl by directly adding an appropriate volume of 5 M NaCl solution. This would not affect the volume in excess, keeping the concentration of other ingredients about the same.
2. Add a volume of 1 M Imidazole pH 7.5 to the final concentration of 10-20 mM.

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 the pellet with detergents known to extract membrane associated proteins
Cell extract is too dilute	Extraction Buffer volume is not optimized	Use less extraction buffer or optimize the volume
Cell extract is too concentrated	Extraction Buffer volume is not optimized	Use more extraction buffer or dilute the cell extract with the buffer
Extracted Proteins are getting degraded	Proteolysis	Use Protease Inhibitor Cocktail (Genesee Cat. No. 18-428) and perform all lysis steps at 4^0C

Related Products

1. **BCA Protein Assay Kit (Cat. No. 18-440 & 18-441)**
   For protein estimation and is also compatible with proteins extracted with Insect Cell Protein Extraction Buffer
2. **Protein Loading Buffer [2X], Cat. No. 20-309**
   Non-reducing ready to use buffer for loading protein samples on to the gel.
3. **Protein Loading Buffer [2X], Cat. No. 20-310**
   Reducing ready to use buffer for loading protein samples on to the gel.