PROMETHEUS™
Protein Biology Products

Instruction Manual

Mammalian Cell Protein Extraction Buffer Catalog No.: 18-406, 18-407 & 18-408

Our mammalian cell protein extraction buffer has been designed for extracting soluble proteins from mammalian cells. The unique proprietary composition of this buffer enables isolation of functionally active proteins without any mechanical treatment such as sonication. The supplied protein extraction buffer contains a proprietary nonionic detergent with salts and organic buffering agents, that enables rapid, mild and efficient lysis of mammalian cells. This unique composition of the buffer offers adaptability for different applications and compatibility with downstream applications, such as reporter assays (e.g., luciferase, β-galactosidase, chloramphenicol acetyltransferase), protein assays (e.g., PKA, PKC, tyrosine kinase), immunoassays (e.g., Western blot, ELISA, RIA) and protein purification. The buffer is also compatible with protease inhibitors, kinase and phosphatase inhibitors, and with BCA protein assays.

Items Supplied:

Name	Cat. No. 18-406	Cat. No. 18-407	Cat. No. 18-408	Storage Condition*
Mammalian Cell Protein Extraction Buffer	250 ml	500 ml	1L	4^0C

**Shipped at ambient temperature and upon receiving, store at 4^0C. The buffer is stable for one year, if stored and used as recommended.*

Additional Materials Needed (Not Supplied):

- Protease inhibitor Cocktail
- Phosphate Buffered Saline (PBS)

Important Details about the Product:

- All steps outlined in the protocol must be followed exactly as indicated to avoid partial lysis of mammalian cells, which adversely affects protein yield.
- Perform all lysis steps at 4^0C or on ice to minimize proteolysis.
- **Adherent Cells vs. Cell Pellets:** The protein extraction buffer effectively lyses both plated cells and cells pelleted from suspension cultures or scrapped cells.
- The protein extraction buffer has been tested on **cell lines** representing several different cell types. Complete lysis of adherent cells is observed with the following cell lines: CHO, COS-7, NIH3T3, Hepa 1-6, 293, MDA, MB 231 and FM2 cells.
- **Protease inhibitors**, such as our Protease Inhibitor Cocktail, Mammalian (Genesee Cat. No. 18-428) may be added to the extraction buffer.
- For immunoassays, such as ELISA or RIA, extracts prepared in this buffer alone generate satisfactory results; however, adding 150mM NaCl to the cell lysate often improves results.
- The **volume** of mammalian extraction buffer should be adjusted depending on the volume of mammalian cells used for a particular experimental condition. For example, 1ml mammalian protein extraction buffer should be used for each 2 x 10^7cells from mammalian cultures with approximate yield of 0.5 mg to 1.5 mg/ml soluble proteins.
- **The volume** of extraction buffer for Cell Lysis indicated in the table below are optimal for maximum cell lysis without scraping cells. If more concentrated extracts are preferred, use a smaller volume; however, scraping the cells is necessary for maximal recovery. If cell volume is unknown, it may be estimated. For example, 2 x 10^6 of HeLa cells equals ~10µl of a packed cell volume, which is equivalent to 20mg of cells and requires 200µl of the protein extraction buffer.

Protocol for Protein Extraction from Monolayer (adherent) Mammalian Cells:

Note: *Mammalian Protein Extraction Buffer does not contain protease inhibitors and a Protease Inhibitor Cocktail, Mammalian (e.g. Genesee Cat. No. 18-428) can be added to the buffer.*

1. Carefully remove/decant culture medium from the adherent cells.

Optional Wash: *If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash cells once PBS (wash buffer).*

2. Add the appropriate volume of Mammalian Protein Extraction Buffer to the plate or to each plate well as per the Table-1 below. Shake the culture plate gently for 5-10 minutes.

Table-1: Suggested volume of protein extraction buffer to use for different sizes of culture plates.

Plate Size/Surface Area	Mammalian Protein Extraction Buffer Volume
100mm*	600-1000 µl
60mm	400-600 µl
6-well plate	300-500 µl per well
24-well plate	150-300 µl per well
96-well plate	75-150 µl per well

*Cells grown in 100mm plates typically contain 10^7 cells (50mg) and yield ~3mg total protein depending on cell type.

3. Collect the lysate and transfer it to a new microcentrifuge tube.
4. Centrifuge the lysate samples at ~14,000 × *g* for 15 minutes to pellet the cell debris.
5. Transfer the supernatant containing mammalian cell proteins to a new tube for further analysis and downstream applications.

Procedure for Protein Extraction from Suspension-cultured Mammalian Cells
Note: *Total protein yield for 100mg of wet cell pellet is approximately 4-5mg depending on cell type.*

1. Pellet the suspension of cells by centrifugation at 2,500 × *g* for 10 minutes. Discard the supernatant.
Optional Wash: *If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash the cells once by resuspending the cell pellet in PBS (wash buffer).*
2. Pellet cells by centrifugation at 2,500 × *g* for 10 minutes.
3. Add Mammalian Protein Extraction Buffer to the cell pellet. For 100 µl cell pellet (~100mg), use 1ml of protein extraction buffer. If a large number of cells are used, first add 1/10 the final recommended volume of protein extraction buffer to the cell pellet. Pipette the mixture up and down to resuspend the pellet. Add the remaining volume of the protein extraction buffer to the cell suspension.
4. Shake mixture gently for 10 minutes. Remove cell debris by centrifugation at ~14,000 × *g* for 15 minutes.
5. Transfer the supernatant containing mammalian cell proteins to a new tube for analysis and downstream applications.

TROUBLESHOOTING

Problem	Possible Cause	Solution
Protein yield is low.	Low protein expression	Optimize the transfection method
	Insufficient volume of Mammalian Protein Extraction Buffer was used	Add more protein extraction buffer
	Protein Extraction Buffer was unable to penetrate the cell membrane	Increase the incubation time and shake more vigorously during incubation
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 our native Protein Extraction Buffers.
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.