INSTRUCTIONS

M-PER® Mammalian Protein Extraction Reagent

PIERCE
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78503 78501 78505 0805.1

Number	Description
78503	**M-PER® Mammalian Protein Extraction Reagent**, 25 ml, sufficient reagent to extract protein from approximately 2.5 g of cells
78501	**M-PER® Mammalian Protein Extraction Reagent**, 250 ml, sufficient reagent to extract protein from approximately 25 g of cells
78505	**M-PER® Mammalian Protein Extraction Reagent**, 1 L, sufficient reagent to extract protein from approximately 100 g of cells

Storage: Upon receipt store product at room temperature.

Introduction

M-PER® Mammalian Protein Extraction Reagent extracts cytoplasmic and nuclear protein from cultured mammalian cells. M-PER® Reagent utilizes a proprietary detergent in 25 mM bicine buffer (pH 7.6) for mammalian cell lysis. The simple composition of this reagent is compatible with many different applications, such as reporter assays (e.g., luciferase, β-galactosidase, chloramphenicol acetyltransferase), protein assays (e.g., PKA, PKC, tyrosine kinase), immunoassays (e.g., Western blots, ELISAs, RIAs) and protein purification. Cell lysis using the M-PER® Reagent results in rapid, mild and efficient lysis. The reagent is dialyzable and the cell lysate is compatible with protein assays such as Coomassie Plus™ and BCA Protein Assays.

Important Product Information

- **Adherent Cells vs. Cell Pellets:** M-PER® Reagent effectively lyses both plated cells and cells pelleted from suspension cultures or scraped cells. For direct, in-the-plate lysis of adherent cells, the efficiency of protein extraction with M-PER® Reagent is similar to freeze/thaw methods. For lysis of pelleted cells either from cell suspension or scraped adherent cells, the efficiency of protein extraction with M-PER® Reagent is typically 25% higher than that achieved with freeze-thaw (three cycles) and 20% higher than sonication (2 minutes with 50% pulse) methods.
- **Cell Lines:** M-PER® Reagent has been tested on cell lines representing several different cell types. Complete lysis of adherent cells is observed with, but is not limited to, the following cell lines: COS-7, NIH 3T3, Hepa 1-6, 293, CHO, MDA, MB 231 and FM2 cells. For protein extraction from tissues, greater efficiency may be achieved using T-PER® Tissue Protein Extraction Reagent (Product No. 78510).
- **Additives:** Protease inhibitors, such as Halt™ Protease Inhibitor Cocktail Kit (Product No. 78410) may be added to the reagent. For immunoassays, such as ELISA or RIA, extracts prepared in M-PER® Reagent alone will generate satisfactory results; however, adding 150 mM NaCl to the cell lysate often improves results.
- **Volume for Cell Lysis:** Volumes indicated in Table 1 are optimal for maximum cell lysis without scraping cells. If more concentrated extracts are preferred, a smaller volume may be used; however, scraping the cells is necessary for maximal recovery. If the volume of cells is unknown, it may be estimated. For example, 2 x10^6 of HeLa cells equals ~10 µl of a packed cell volume, which is equivalent to 20 mg of cells and requires 200 µl of M-PER® Reagent.
- **Compatibility with Protein Assays:** M-PER® Reagent is compatible with Coomassie Plus™ Protein Assay (Protein No. 23236) and BCA Protein Assay Kit (Product No 23225).

Procedure for Lysis of Adherent Mammalian Cells

Note: M-PER® Reagent does not contain protease inhibitors. Halt™ Protease Inhibitor Cocktail Kit (Product No. 78410) may be added to the reagent if desired.

1. Carefully remove (decant) culture medium from the adherent cells.

   Note: If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash cells once in wash buffer (e.g., PBS).

2. Add the appropriate amount of M-PER® Reagent to the plate or to each plate well (see Table 1). Shake gently for 5 minutes.

Table 1. Suggested volume of M-PER® Reagent to use for different sizes of standard culture plates.

Plate Size/Surface Area	Volume of M-PER® Reagent
100 mm*	500-1,000 µl
60 mm	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

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

Note: Cell debris may be removed as described in Steps 3-4 or, if applicable, the lysate may be used directly for analysis in the presence of the cell debris.

3. Collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at ~14,000 x *g* for 5-10 minutes to pellet the cell debris.
4. Transfer the supernatant to a new tube for further analysis.

Procedure for Lysis of Mammalian Cells in Suspension

1. Pellet the suspension of cells by centrifugation at 2,500 x *g* for 10 minutes. Discard the supernatant.
2. 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 wash buffer (e.g., PBS). Pellet the cells by centrifugation at 2,500 x *g* for 10 minutes.
3. Add M-PER® Reagent to the cell pellet. Use at least 1 ml of M-PER® Reagent for each 100 mg (~100 µl) of wet cell pellet. If a large amount of cells is used, first add 1/10 the final recommended volume of M-PER® Reagent to the cell pellet. Pipette the mixture up and down to resuspend the pellet. Add the rest of the M-PER® Reagent to the cell suspension.

   Note: Total protein yield for 100 mg of wet cell pellet is approximately 6 mg depending on cell type.

4. Shake mixture gently for 10 minutes. Remove cell debris by centrifugation at ~14,000 x *g* for 15 minutes.
5. Transfer the supernatant to a new tube for further analysis.

Troubleshooting

Problem	Possible Cause	Solution
Low protein yield	Protein expression is low	Optimize the transfection procedure
	Insufficient amount of M-PER® Reagent was used	Add more M-PER®Reagent
	M-PER® Reagent was unable to penetrate the cell membrane	Increase incubation time and shake more vigorously during incubation
Unable to retrieve membrane protein	M-PER® Reagent is for the extraction of nuclear and cytoplasmic protein	Use MEM-PER® Membrane Protein Extraction Reagent (Product No. 23236)

Related Pierce Products

78410	**Halt™ Protease Inhibitor Cocktail Kit**
78248	**B-PER® Bacterial Protein Extraction Reagent,** 500 ml
78990	**Y-PER® Yeast Protein Extraction Reagent,** 500 ml
89826	**Mem-PER® Membrane Protein Extraction Reagent Kit**
23236	**Coomassie Plus™ Protein Assay Kit**
23227	**BCA Protein Assay Kit**
78833	**NE-PER® Nuclear and Cytoplasmic Extraction Kit**
45335	**Seize® Primary Immunoprecipitation Kit**
34080	**SuperSignal® West Pico Chemiluminescent Substrate,*** 500 ml, Western blot substrate for HRP
34076	**SuperSignal® West Dura Extended Duration Substrate,*** 200 ml, Western blot substrate for HRP

Product References

Campa, M.J., *et al.* (2003). Protein expression profiling identifies macrophage migration inhibitory factor and cyclophilin A as potential molecular targets in non-small cell lung cancer. *Cancer Res.* **63:**1652-6.

Deng, W., *et al.* (2003). LPA protects intestinal epithelial cells from apoptosis by inhibiting the mitochondrial pathway. *Amer. J. Physiol-Gastrointest. L.* **284:**821-9.

Phiel, C.J., *et al.* (2001). Differential binding of an SRF/NK-2/MEF2 transcription factor complex in normal versus neoplastic smooth muscle tissues. *Biol. Chem.* **276(37):**34637-50.

Waite, K.A. and Eng, C. (2003). BMP2 exposure results in decreased PTEN protein degradation and increased PTEN levels. *Hum. Mol. Genet.* **12(6):**679-84.

*SuperSignal® Technology is protected by U.S. Patent #6,432,662.