INSTRUCTIONS

M-PER® Mammalian Protein Extraction Reagent

78501 78503 78505

0805.3

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 using a proprietary detergent in 25 mM bicine buffer (pH 7.6). 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 blot, ELISA, RIA) and protein purification. M-PER Reagent enables rapid, mild and efficient lysis. The reagent is dialyzable and the cell lysate is compatible with protein assays such as Coomassie Plus – The Better Bradford™ Assay and the Pierce® BCA Protein Assay.

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, protein extraction efficiency using M-PER Reagent is similar to freeze/thaw methods. For lysis of pelleted cells, either from cell suspension or scraped adherent cells, protein extraction efficiency 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 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, use a smaller volume; however, scraping the cells is necessary for maximal recovery. If cell volume is unknown, it may be estimated. For example, 2×10^6 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 – The Better Bradford Assay (Protein No. 23236) and the Pierce BCA Protein Assay Kit (Product No 23225).

Procedure for Lysis of Monolayer-cultured Mammalian Cells

Note: M-PER Reagent does not contain protease inhibitors. If desired, add Halt Protease Inhibitor Cocktail Kit (Product No. 78410) to the reagent.

1. Carefully remove (decant) culture medium from 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	M-PER Reagent Volume
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.

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

Procedure for Lysis of Suspension-cultured Mammalian Cells

1. Pellet the suspension of cells by centrifugation at 2,500 × *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 cells by centrifugation at 2,500 × *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 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 × *g* for 15 minutes.
5. Transfer the supernatant to a new tube for 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 extracts nuclear and cytoplasmic protein	Use Mem-PER® Membrane Protein Extraction Reagent (Product No. 89826)

Related 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 – The Better Bradford Assay**
23227	**Pierce 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.

B-PER® Technology is protected by U.S. Patent # 6,174,704.

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

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