INSTRUCTIONS

M-PER® Mammalian Protein Extraction Reagent

78501 78503 78505

0805.5

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

Storage: Upon receipt store product at room temperature. Product shipped at ambient temperature.

Introduction

The Thermo Scientific M-PER Mammalian Protein Extraction Reagent extracts cytoplasmic and nuclear protein from cultured mammalian cells using a proprietary detergent in 25mM 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 the Thermo Scientific Coomassie Plus (Bradford) Assay and the Thermo Scientific 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-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, NIH3T3, Hepa 1-6, 293, CHO, MDA, MB 231 and FM2 cells. For protein extraction from tissues, greater efficiency may be achieved using Thermo Scientific T-PER Tissue Protein Extraction Reagent (Product No. 78510).
- **Additives:** Protease inhibitors, such as Thermo Scientific Halt Protease Inhibitor Cocktail, EDTA-Free (Product No. 87785) 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 150mM 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 ~10µL of a packed cell volume, which is equivalent to 20mg of cells and requires 200µL of M-PER Reagent.
- **Compatibility with Protein Assays:** M-PER Reagent is compatible with the Coomassie Plus (Bradford) Protein Assay Kit (Product No. 23236) and the Pierce® BCA Protein Assay Kit (Product No 23225).

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Procedure for Lysis of Monolayer-cultured Mammalian Cells

Note: M-PER Reagent does not contain protease inhibitors. If desired, add Halt™ Protease Inhibitor Cocktail, EDTA-Free (Product No. 87785) 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 Thermo Scientific M-PER Reagent to use for different sizes of standard culture plates.

Plate Size/Surface Area	M-PER Reagent Volume
100mm*	500-1000µL
60mm	250-500µL
6-well plate	200-400µL per well
24-well plate	100-200µL per well
96-well plate	50-100µL 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 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 2500 × *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 2500 × *g* for 10 minutes.
3. Add M-PER Reagent to the cell pellet. Use at least 1mL of M-PER Reagent for each 100mg (~100µL) 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 100mg of wet cell pellet is approximately 6mg 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 was 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 only nuclear and cytoplasmic proteins	Use Thermo Scientific Mem-PER Membrane Protein Extraction Reagent (Product No. 89826)

Related Thermo Scientific Products

87785	**Halt Protease Inhibitor Cocktail, EDTA-Free** (100X), 1mL
87786	**Halt Protease Inhibitor Cocktail,** contains sufficient reagents to treat 100mL of sample
78248	**B-PER® Bacterial Protein Extraction Reagent,** 500mL
78990	**Y-PER® Yeast Protein Extraction Reagent,** 500mL
89826	**Mem-PER® Membrane Protein Extraction Reagent Kit**
23236	**Coomassie Plus (Bradford) Protein Assay Kit**
23227	**Pierce BCA Protein Assay Kit**
78833	**NE-PER® Nuclear and Cytoplasmic Extraction Kit**
26148	**Pierce Direct IP Kit**
34080	**SuperSignal® West Pico Chemiluminescent Substrate,** 500mL, Western blot substrate for HRP
34076	**SuperSignal West Dura Extended Duration Substrate,** 200mL, 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.

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

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce