INSTRUCTIONS

M-PER® Mammalian Protein Extraction Reagent

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>78503</td>
<td>M-PER Mammalian Protein Extraction Reagent, 25 ml, sufficient reagent to extract protein from approximately 2.5 g of cells</td>
</tr>
<tr>
<td>78501</td>
<td>M-PER Mammalian Protein Extraction Reagent, 250 ml, sufficient reagent to extract protein from approximately 25 g of cells</td>
</tr>
<tr>
<td>78505</td>
<td>M-PER Mammalian Protein Extraction Reagent, 1 L, sufficient reagent to extract protein from approximately 100 g of cells</td>
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</table>

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 lyases 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.
   
<table>
<thead>
<tr>
<th>Plate Size/Surface Area</th>
<th>M-PER Reagent Volume</th>
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</thead>
<tbody>
<tr>
<td>100 mm*</td>
<td>500-1,000 µl</td>
</tr>
<tr>
<td>60 mm</td>
<td>250-500 µl</td>
</tr>
<tr>
<td>6-well plate</td>
<td>200-400 µl per well</td>
</tr>
<tr>
<td>24-well plate</td>
<td>100-200 µl per well</td>
</tr>
<tr>
<td>96-well plate</td>
<td>50-100 µl per well</td>
</tr>
</tbody>
</table>
   
   *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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low protein yield</td>
<td>Protein expression is low</td>
<td>Optimize the transfection procedure</td>
</tr>
<tr>
<td>Insufficient amount of M-PER Reagent was used</td>
<td>Add more M-PER Reagent</td>
<td></td>
</tr>
<tr>
<td>M-PER Reagent was unable to penetrate the cell membrane</td>
<td>Increase incubation time and shake more vigorously during incubation</td>
<td></td>
</tr>
<tr>
<td>Unable to retrieve membrane protein</td>
<td>M-PER Reagent extracts nuclear and cytoplasmic protein</td>
<td>Use Mem-PER® Membrane Protein Extraction Reagent (Product No. 89826)</td>
</tr>
</tbody>
</table>
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


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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