**INTRODUCTION**
SoluLyse-M™ Mammalian Protein Extraction Reagent provides a highly efficient yet gentle method for extracting proteins from mammalian cells. It extracts cytoplasmic and nuclear protein from cultured cell lines using a proprietary non-ionic detergent in 25mM Tris, pH 7.4 and 250mM Sucrose. SoluLyse-M™ Mammalian Protein Extraction Reagent is compatible with many different applications, such as reporter assays, immunassays and protein purification. The reagent is easily dialyzable and compatible with protein assays such as Western blotting, Coomassie Blue, Bradford and the BCA™ Protein Assays.

**PROTOCOLS FOR LYSIS OF ADHERENT MAMMALIAN CELLS**

1. Carefully remove the culture medium from adherent cells.
   **NOTE:** if the culture medium contains phenol red or other reagents that may interfere with subsequent protein analysis, wash cells once with PBS.

2. Add the appropriate amount of SoluLyse-M™ Reagent to the plate or to each plate well according to Table 1.
   **NOTE:** SoluLyse-M™ Reagent does not contain protease inhibitors. You may add protease inhibitors if desired.

   **Table 1. Suggested volume of SoluLyse-M™ Reagent to use for different sizes of standard culture plates**

<table>
<thead>
<tr>
<th>Plate Type</th>
<th>Volume of SoluLyse-M™ Reagent</th>
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<tbody>
<tr>
<td>100mm*</td>
<td>500-1000 µl per well</td>
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<tr>
<td>60mm</td>
<td>250-500 µl per well</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>
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</tbody>
</table>

   *Typically, 100mm plates have a cell density of 10⁷ cells (50mg) and yield of approximately 3mg of total protein depending on the cell types.

3. Incubate for 10 minutes at 25°C with gentle agitation or rotation.
   **NOTE:** Some cell types and cells transfected with high amount of DNA and transfection reagent may require a single freeze for 10 minutes at -80°C and thaw at 25°C while gently rotating. We recommend always verifying complete lysis under a light microscope.

4. Collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at 14,000 x g for 5 minutes to pellet the cell debris.
   **NOTE:** Steps 4-5 are optional. The lysate may be used directly for analysis in the presence of the cell debris.

5. Transfer the clarified lysate to a fresh tube for further analysis.

**PROTOCOLS FOR LYSIS OF ADHERENT MAMMALIAN CELLS**

1. Pellet the cell suspension by centrifugation at 3,000 x g for 10 minutes. Carefully remove the supernatant and discard.

2. Optional Wash: If the culture medium contains phenol red or other reagents that may interfere with subsequent protein analysis, wash cells once with PBS. Pellet the washed cells by centrifugation at 3,000 x g for 10 minutes. Discard the supernatant.

3. Add SoluLyse-M™ Reagent to the cell pellet. Use 1ml of SoluLyse-M™ Reagent for each 100mg (approximately 100 ml) of wet cell pellet. If larger amounts of cells are used, first add 1/10 the final recommended volume of SoluLyse-M™ Reagent and resuspend the cell pellet by pipetting. Add the remaining amount of SoluLyse-M™ Reagent to the cell suspension.

4. Shake or rotate the cell suspension gently for 10 minutes. Remove cell debris by centrifugation at 3,000 x g for 15 minutes.

5. Transfer the supernatant to a fresh tube for further analysis.