Introduction

The Thermo Scientific RIPA buffer is one of the most reliable buffers used to lyse cultured mammalian cells from both plated cells and cells pelleted from suspension cultures. This buffer enables protein extraction from cytoplasmic, membrane and nuclear proteins and is compatible with many applications, including reporter assays, protein assays, immunoassays and protein purification.

Important Product Information

- RIPA Buffer does not contain protease or phosphatase inhibitors. If desired, add protease inhibitors, such as Thermo Scientific Halt Protease Inhibitor Cocktail (Product No. 78410) and Halt™ Phosphatase Inhibitor Cocktail (Product No. 78420) to the reagent to prevent proteolysis and maintain phosphorylation status of proteins. Add protease and phosphatase inhibitors immediately before use.

- Use 1mL of cold RIPA Buffer for every 5×10⁶ of HeLa or A431 cells (~20µL of packed cells, which is equivalent to ~40mg of cells). To obtain concentrated protein extracts, directly lyse cells on plate and use less buffer.

- Some protein kinases and other enzymes may be sensitive to the components of the RIPA Buffer, resulting in their decreased activity. In such cases, prepare a RIPA buffer that does not contain sodium deoxycholate and SDS.

- RIPA Buffer is compatible with the Thermo Scientific Pierce BCA Protein Assay Kit (Product No 23225).

Procedure for Lysis of Monolayer-cultured Mammalian Cells

Note: If desired, add protease and phosphatase inhibitors to the RIPA Buffer immediately before use.

1. Carefully remove (decant) culture medium from adherent cells.
2. Wash cells twice with cold PBS.
3. Add cold RIPA Buffer to the cells. Use 1mL of buffer per 75cm² flask containing 5 × 10⁶ HeLa or A431 cells. Keep on ice for 5 minutes, swirling the plate occasionally for uniform spreading.
4. Gather the lysate to one side using a cell scraper, collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at ~14,000 × g for 15 minutes to pellet the cell debris.
   Note: To increase yields, sonicate the pellet for 30 seconds with 50% pulse.
5. Transfer supernatant to a new tube for further analysis.
Procedure for Lysis of Suspension-cultured Mammalian Cells

**Note:** If desired, add protease and phosphatase inhibitors to the RIPA Buffer immediately before use.

1. Pellet the cells by centrifugation at $2500 \times g$ for 5 minutes. Discard the supernatant.
2. Wash cells twice in cold PBS. Pellet cells by centrifugation at $2500 \times g$ for 5 minutes.
3. Add RIPA Buffer to the cell pellet. Use 1mL of RIPA buffer for 40mg (~$5 \times 10^6$ of HeLa cells) of wet cell pellet. Pipette the mixture up and down to suspend the pellet.
   **Note:** To increase yields, sonicate the pellet for 30 seconds with 50% pulse.
4. Shake mixture gently for 15 minutes on ice. Centrifuge mixture at ~$14,000 \times g$ for 15 minutes to pellet the cell debris.
5. Transfer supernatant to a new tube for further analysis.

**Troubleshooting**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low total protein yield</td>
<td>Some cells are more resistant to lysis than others</td>
<td>Make sure the cell pellet is thoroughly suspended in RIPA Buffer and incubate for longer with occasional swirling – sonicate the pellet to increase yield</td>
</tr>
<tr>
<td>Low concentration of proteins</td>
<td>Excess buffer used</td>
<td>Use less buffer (e.g., 0.25-0.5mL per 75cm² flask containing 5 x 10⁶ cells) – use a sufficient amount to cover the entire plate</td>
</tr>
<tr>
<td>Proteolysis</td>
<td>No protease inhibitors added</td>
<td>Add Halt Protease Inhibitor Cocktail to the buffer before use</td>
</tr>
<tr>
<td>Low phosphorylation of proteins</td>
<td>Phosphatase activity</td>
<td>Add Halt Phosphatase Inhibitor Cocktail to the buffer before use</td>
</tr>
<tr>
<td></td>
<td>Protein is non-phosphorylated or poorly phosphorylated</td>
<td>None</td>
</tr>
</tbody>
</table>

**Related Thermo Scientific Products**

- 78410 Halt Protease Inhibitor Cocktail Kit
- 78420 Halt Phosphatase Inhibitor Cocktail, 1mL
- 78248 B-PER® Bacterial Protein Extraction Reagent, 500mL
- 78990 Y-PER® Yeast Protein Extraction Reagent, 500mL
- 89826 Mem-PER® Membrane Protein Extraction Reagent Kit
- 78833 NE-PER® Nuclear and Cytoplasmic Extraction Kit
- 23227 Pierce® BCA Protein Assay Kit
- 26148 Pierce Direct IP Kit
- 34080 SuperSignal® West Pico Chemiluminescent Substrate, 500mL
- 34076 SuperSignal® West Dura Extended Duration Substrate, 200mL

**General References**


This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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