INSTRUCTIONS

# RIPA Buffer

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## 89900 89901

1782.1

| Number | | Description |
|---|---|---|
| **89900** | Buy Now | **RIPA Buffer,** 100mL |
| **89901** | Buy Now | **RIPA Buffer,** 250mL |

Contents: 25mM Tris•HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS

**Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

## 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 $5 \times 10^6$ 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 75cm$^2$ flask containing $5 \times 10^6$ 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 × *g* for 5 minutes. Discard the supernatant.
2. Wash cells twice in cold PBS. Pellet cells by centrifugation at 2500 × *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 × *g* for 15 minutes to pellet the cell debris.
5. Transfer supernatant to a new tube for further analysis.

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Low total protein yield | Some cells are more resistant to lysis than others | Make sure the cell pellet is thoroughly suspended in RIPA Buffer and incubate for longer with occasional swirling – sonicate the pellet to increase yield |
| Low concentration of proteins | Excess buffer used | Use less buffer (e.g., 0.25-0.5mL per 75cm$^2$ flask containing $5 \times 10^6$ cells) – use a sufficient amount to cover the entire plate |
| Proteolysis | No protease inhibitors added | Add Halt Protease Inhibitor Cocktail to the buffer before use |
| Low phosphorylation of proteins | Phosphatase activity | Add Halt Phosphatase Inhibitor Cocktail to the buffer before use |
| | Protein is non-phosphorylated or poorly phosphorylated | None |

## 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

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Pfrepper, K.I. and Flugel R.M. (2005). Molecular characterization of proteolytic processing of the gap proteins of human spumaretrovirus. *Methods in Mol Biol* **304:**435-44.

Sefton, B.M. (2005). Labeling cultured cells with 32Pi and preparing cell lysates for immunoprecipitation. Unit 18.2. F. M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl (eds.) *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.

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