# RIPA Cell Lysis Buffer(1X) with EDTA

**R4100**

## Storage

Store at 2-8°C

Upon receipt, store at 4°C. Product shipped at ambient temperature.

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## Introduction

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 Notes

- RIPA Buffer does not contain protease or phosphatase inhibitors. If desired, add protease inhibitors, such as Xpert Protease Inhibitor Cocktail Solution(100X) (Cat. No. P3100) and Xpert Phosphatase Inhibitor Cocktail Solution(100X) (Cat. No. P3200) to the reagent to prevent proteolysis and maintain phosphory -lation 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 (~ 20 µL of packed cells, which is equivalent to ~40 mg 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 BCA Protein Assay Kit (Cat. No. P8100).

## Composition

Tris-HCl, pH 7.4, 50mM
NaCl , 150 mM
Triton X-100. 1%
Sodium deoxycholate, 0.5%
SDS, 0.1%
EDTA , 2mM

## Protocol : Lyse Monolayer-cultured mammalian cells

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 1 mL of buffer per 75 $cm^2$ flask containing $5 \times 10^6$ HeLa or A431 cells. Keep on ice for 5 minutes, swirling the plate occasionally for uniform spreading.

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

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 collect 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.

## Protocol : Suspension-cultured mammalian cells

1. Collect cells by centrifugation at 2500 × g for 5 minutes. Discard the supernatant.
2. Wash cells twice in cold PBS. Collect cells by centrifugation at 2500 × g for 5 minutes.
3. Add RIPA Buffer to the cell pellet. Use 1 mL of RIPA buffer for 40 mg ($5 \times 10^6$ of HeLa cells) of wet cell pellet. Pipette the mixture up and down to suspend the pellet.

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

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.

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