PROMETHEUS™
Protein Biology Products

**Instruction Manual**

## RIPA Lysis & Extraction Buffer — Catalog No.: 18-415, 18-416 & 18-417

### Introduction

Our RIPA; Radio-Immunoprecipitation Assay Lysis & Extraction Buffer is a complete cell lysis solution, used for rapid and efficient cell lysis and solubilization of proteins from both adherent and suspension cultured mammalian cells. The buffer is very effective in extracting cytoplasmic, nuclear and membrane proteins and the time for protein extraction takes about 60 minutes. RIPA lysis & extraction buffer contains non-ionic and ionic detergents which are able to extract proteins from different cell types and membrane structures. The supplied RIPA buffer contains 1% NP-40, 1% sodium deoxycholate, 0.1% SDS with 25mM Tris and 150mM NaCl. RIPA buffer ensures efficient cell lysis and protein solubilization preventing protein degradation and interference with protein immunoreactivity and biological activity. Since most antibodies and protein antigens are not adversely affected by the components of this solution, proteins extracted with RIPA buffer is compatible with various downstream application, such as immunoprecipitation and molecular pull-down assays, including reporter assays, protein assays, immunoassays and protein purification. RIPA buffer minimizes non-specific protein-binding interactions and keep low background, while allowing most specific interactions to occur, enabling studies of relevant protein-protein interactions.

### Buffer Components

| Name | Cat. No. 18-415 | Cat. No. 18-416 | Cat. No. 18-417 | Storage Temp* |
|---|---|---|---|---|
| RIPA Lysis & Extraction Buffer | 125 ml | 250 ml | 500 ml | $4^0$C to RT |

**The RIPA Lysis & Extraction Buffer is shipped at ambient temperature. Upon receiving, store at $4^0$C or Room Temp. If precipitate develops after storing at $4^0$C, bring it to solution at room temperature before use.*

**IMPORTANT NOTE ABOUT THE PRODUCT AND ITS USAGE:**

- Our RIPA Lysis & Extraction Buffer is supplied as ready-to-use solution and requires no further dilution (contains 1% NP-40, 1% sodium deoxycholate, 0.1% SDS,150mM NaCl and 25mM Tris-HCl, pH 7.6).
- If desired, add protease inhibitor and phosphatase inhibitor cocktails to the RIPA lysis buffer for preventing proteolysis and maintaining the phosphorylation of proteins.
- RIPA Lysis Buffer does not contain protease or phosphatase inhibitors in it. If required, add a Protease Inhibitor Cocktail (Cat. No. 18-421 or 18-428) and Phosphatase Inhibitor Cocktail into the RIPA Buffer to prevent proteolysis and maintaining the phosphorylation of proteins. Add protease and phosphatase inhibitors immediately before use.
- Use 1mL of cold RIPA Buffer for every 5 x $10^6$ of HeLa or A431 cells (~20µl of packed cells, which is equivalent to ~40mg of cells).
- Some protein kinases and other enzymes may be sensitive to the chemicals present in the RIPA Lysis Buffer, resulting in their decreased activity. In such cases a modified RIPA buffer that does not contain sodium deoxycholate and SDS is needed.
- RIPA Lysis & Extraction Buffer is compatible with Bicinchoninic Acid (BCA) Protein Assay Kit (Cat. No. 18-440). Bradford Protein Assay kit is not recommended.
- All steps of cell lysis and protein extraction are required to be performed on ice or at 4˚C.

**Items Needed, But Not Supplied with the Product**

The supplied product contains only RIPA Lysis & Extraction Buffer and following items may be needed, depending on the experiment:

- Phosphate buffered saline (PBS)
- Protease inhibitor cocktail (Cat. No. 18-421 or 18-428) and phosphatase inhibitors/cocktail
- 2 ml Microcentrifuge tubes
- Tissue homogenizer
- Microcentrifuge
- Cell scraper for adherent cells

**PROTOCOLS**

**Procedure for Lysis of Monolayer-cultured Adherent Mammalian Cells:**
***Note:*** *Pre-chill an appropriate volume of RIPA Lysis Buffer at 4˚C, if it was stored at RT. If desired, add protease inhibitor and/or phosphatase inhibitor cocktail into the lysis buffer immediately before using the buffer.*

1. In a microcentrifuge tube, resuspend $5\times10^6$ cells in the growth media by scraping the cells off the surface of the plate with a cell scraper. Centrifuge harvested cell suspension at 600xg for 5min, then carefully remove and discard the supernatant.
2. Resuspend the cells in chilled PBS. Centrifuge at 600xg for 5min, then carefully remove and discard the supernatant.
3. Add 0.5 ml of chilled RIPA lysis buffer to the cell pellet. Vortex briefly and incubate on ice for 30 minutes.
4. Centrifuge at 14000xg for 10 minutes and transfer the supernatant to a new tube for further analysis and downstream applications.
5. The extracted proteins can be used immediately or stored frozen at -20°C until needed. Prepare small aliquots to avoid repeated freeze-thaw cycles, which may degrade the sample proteins.

**Procedure for Lysis of Suspension-cultured Mammalian Cells:**
***Note:*** *Pre-chill an appropriate volume of RIPA Lysis Buffer at 4˚C, if it was stored at RT. If desired, add protease inhibitor and phosphatase inhibitor to the lysis buffer immediately before use.*

1. In a microcentrifuge tube, harvest 5×106 cells by centrifugation at 600xg for 5min. Carefully remove and discard the supernatant.
2. Resuspend the cells in chilled PBS. Centrifuge at 600xg for 5min, then carefully remove and discard the supernatant.
3. Add 0.5 ml of chilled RIPA lysis buffer to the cell pellet. Vortex briefly. Incubate on ice for 30 minutes.
4. Centrifuge samples at 14000xg for 10 minutes and transfer the supernatant to a new tube for further analysis and downstream applications.
5. The extracted proteins can be used immediately or stored frozen at -20°C until needed. Prepare small aliquots to avoid repeated freeze-thaw cycles, which may degrade the sample proteins.

Procedure for Lysis of Tissues:
***Note:*** *Pre-chill an appropriate volume of RIPA Lysis Buffer at 4˚C, if was stored at RT. If desired, add protease inhibitor and/or phosphatase inhibitor cocktail(s) to the lysis buffer immediately before use.*

1. Place the fresh tissue into chilled PBS and rinse several times. Mince the tissue into small pieces.
2. Add chilled RIPA Lysis Buffer to the tissue at 10ml per 1g tissue. Use a smaller volume of reagent if a more concentrated protein extract is required.
3. Homogenize for several minutes at high speed until no tissue chunks remain.
4. Incubate on ice for 30 minutes.
5. Centrifuge at ~10000xg for 10 minutes and transfer the supernatant to a new tube for further analysis and downstream applications.
6. The extracted proteins can be used immediately or stored frozen at -20°C until needed. Prepare small aliquots to avoid repeated freeze-thaw cycles, which may degrade the sample proteins.

**TROUBLESHOOTING**

| Problem | Possible Cause | Solution |
|---|---|---|
| Low protein yield | Cells were not completely lysed.<br><br>Too few cells per volume of RIPA Cell Lysis Buffer were used. | Resuspend cells completely in RIPA Cell Lysis Buffer and increase incubation time on ice. Brief sonication at 50% pulse may also increase yield.<br>Increase the number of cells or decrease volume of RIPA Cell Lysis Buffer. |
| Protein samples degraded | Samples underwent proteolysis. | Use protease inhibitors cocktail and keep sample on ice at all times. |
| Low phosphorylation of proteins | Phosphatase activity present in the sample | Use Phosphatase Inhibitor Cocktail and keep the sample on ice throughout the procedure |