

## Product Information

### RIPA Buffer

Product Number **R0278**

Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

Extraction of cellular proteins requires efficient cell lysis and protein solubilization, while avoiding protein degradation and/or interference with protein immunoreactivity and biological activity.

RIPA (**R**adio-**I**mmunoprecipitation **A**ssay) Buffer enables rapid, efficient cell lysis and solubilization of proteins from both adherent and suspension cultured mammalian cells. It has long been a widely used lysis and wash buffer for small-scale affinity pull-down applications, such as immunoprecipitation, since most antibodies and protein antigens are not adversely affected by the components of this buffer.<sup>1</sup> In addition, RIPA Buffer minimizes non-specific protein-binding interactions to keep background low, while allowing most specific interactions to occur, enabling studies of relevant protein-protein interactions.

RIPA Buffer is supplied as a ready to use solution that requires no preparation. Protease and phosphatase inhibitors may be added to the lysis buffer as needed. One ml of the RIPA Buffer is sufficient to lyse cells from one 100 mm culture dish (0.5 to  $5 \times 10^7$  cells) of most adherent mammalian cell lines.

### Components

RIPA Buffer (Product Number R0278) –  
50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate.<sup>1</sup>

### Reagents and Equipment Required but Not Provided.

(Product Numbers are given where appropriate)

- Centrifuge
- Appropriate centrifuge tubes
- A physiological wash buffer (e.g., Dulbecco's Phosphate Buffered Saline [DPBS], Product Number D8662)

- Cell scrapers for adherent cells
- Protease Inhibitor Cocktail for Mammalian Tissues (Product Number P8340)
- Phosphatase Inhibitor Cocktails
  - for Serine/Threonine Phosphatases and L-Isozymes of Alkaline Phosphatases (Product Number P2850)
  - for Tyrosine Protein Phosphatases, Acid and Alkaline Phosphatases (Product Number P5726)

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

It is recommended to store the product at 2–8 °C.

### Procedure

#### A. Procedure for lysis of adherent cells

1. Remove growth medium from the cells by decantation or aspiration.
2. Wash cells to remove residual medium. Slowly add a volume of a physiological wash solution, such as DPBS, equal to the original medium volume being careful not to dislodge cells. Mix gently and remove the wash solution. Repeat the wash once in order to remove any other minor contaminants. More washing steps can be done, but two is usually sufficient to remove nearly all of the contaminants.
3. After removal of the final wash solution from the cells, add an appropriate volume of RIPA Buffer (1 ml for  $0.5$  to  $5 \times 10^7$  cells). Incubate on ice or in a refrigerator (2–8 °C) for five minutes.

4. Rapidly scrape the plate with a cell scraper to remove and lyse residual cells. Transfer the cell lysate to a tube on ice. The lysate can either be used immediately or quick-frozen in liquid nitrogen and stored at  $-70\text{ }^{\circ}\text{C}$  for future use. It is best to freeze the lysate before clarification, since the freeze-thaw cycle may cause some denatured protein aggregates.

5. Clarify the lysate by centrifugation at  $8,000 \times g$  for 10 minutes at  $4\text{ }^{\circ}\text{C}$  to pellet the cell debris.

Note: If a mucoid aggregate of denatured nucleic acids is present, carefully remove it with a micropipette before centrifugation.

6. Carefully transfer the supernatant containing the soluble protein to a tube on ice for immunoprecipitation or other analysis.

#### B. Procedure for lysis of suspension cultured cells

1. Pellet the cells by centrifugation at  $450 \times g$  for 5 minutes.

2. Carefully remove the medium from the cell pellet by decantation or aspiration.

3. Wash the cells to remove residual medium. Add a volume of a physiological wash solution, such as DPBS, equal to the original medium volume. Mix or vortex briefly to resuspend the cells completely. Centrifuge for 5 minutes at  $450 \times g$  to pellet the cells and carefully remove wash solution supernatant. Repeat the wash once in order to remove any other minor contaminants. More washing steps can be done, but two is usually sufficient to remove nearly all of the contaminants.

4. After removal of the final wash solution from the cells, add an appropriate volume of RIPA Buffer (1 ml per  $0.5$  to  $5 \times 10^7$  cells) to the cell pellet, and mix or vortex briefly to resuspend the cells completely. Incubate on ice or in a refrigerator ( $2$ – $8\text{ }^{\circ}\text{C}$ ) for five minutes. Vortex briefly to resuspend and lyse residual cells.

5. The lysate can either be used immediately or quick-frozen in liquid nitrogen and stored at  $-70\text{ }^{\circ}\text{C}$  for future use.

6. Clarify the lysate by centrifugation at  $8,000 \times g$  for 10 minutes at  $4\text{ }^{\circ}\text{C}$  to pellet the cell debris.

Note: If a mucoid aggregate of denatured nucleic acids is present, carefully remove it with a micropipette before centrifugation.

7. Carefully transfer the supernatant containing the soluble protein to a tube on ice for immunoprecipitation or other analysis.

#### **References**

1. Antibodies a Laboratory Manual, Harlow, E. and Lane, D., Cold Spring Harbor Press (Cold Spring Harbor, NY: 1988), p 449.

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