

### **RIPA Buffer**

Cat No. RIP-M-100

#### **DESCRIPTION**

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.

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 Buffer enables rapid, efficient cell lysis and solubilization of protein 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 the this buffer. In addition, RIPA buffer minimizes non-specific protein-binding most specific interactions to occur, enabling studies of relevant protein-protein interactions.

RIPA buffer is supplied as several kinds of solution. 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 100mm culture dish (0.5 to  $5 \times 10^7$  cells) of most adherent mammalian cell lines.

#### **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 (1ml 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°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 x g for 10 minutes at 4°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 x 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 x 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 (1ml per 0.5 to  $5 \times 10^7$  cells) to the cell pellet, and mix or vortex briefly to resuspend the cells completely.

# Technical Data Sheet

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Incubate on ice or in a refrigerator (2-8°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°C for future use.

6. Clarify the lysate by centrifugation at 8,000 X g for 10 minutes at 4°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

## **REFERENCE**

Antibodies a Laboratory Manual, Harlow, E. and Lane, D., Cold Spring Harbor Press (Cold Spring Harbor, NY: 1988), p449.

## **STORAGE**

Store at 2-8°C.

For laboratory use only. Not intended for human therapeutic or diagnostic use.

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