PROMPT
Proteins for Research

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RIPA Buffer

R0020

Introduction

RIPA (Radio-Immunoprecipitation Assay) solution is a ready to use complete cell lysis buffer for rapid and efficient cell lysis and solubilization of proteins from both adherent and suspension cultured mammalian cells. It contains ionic and non-ionic detergents which enables the extraction of membrane, nuclear and cytoplasmic proteins. The buffer minimizes non-specific protein-binding interactions to keep the background low, while allowing most specific interactions to occur. RIPA buffer-conducted protein extraction is compatible with various downstream immunoprecipitation and molecular pull-down assays, including reporter assays, protein assays, immunoassays and protein purification.

Size

1 × 50mL

Form

Colorless liquid

Storage

Store at 4°C. The buffer is stable for 12 months.

Recommended working concentration:

- 1mL RIPA Buffer per 5×10^6 cells in suspension
- 1mL RIPA Buffer per 5×10^6 adherent mammalian cells

Protocol

Lysis of monolayer-cultured mammalian cells

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

- Carefully remove culture medium from adherent cells.
- Wash cells twice with cold PBS.
- Add cold RIPA Buffer to the cells. Use 1 mL of buffer per 75 cm^2 flask containing 5×10^6 HeLa or A431 cells. Keep on ice for 5 minutes, swirling the plate occasionally for uniform spreading.
- 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.
- Transfer supernatant to a new tube for further analysis.

Lysis of suspension-cultured mammalian cells

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

- Collect cells by centrifugation at 2500 ×g for 5 minutes. Discard the supernatant.
- Wash cells twice in cold PBS. Collect cells by centrifugation at 2500 ×g for 5 minutes.
- Add RIPA Buffer to the cell pellet. Use 1 mL of RIPA Buffer for 40 mg (~5×10^6 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.
- Shake mixture gently for 15 minutes on ice. Centrifuge mixture at ~14,000 × g for 15 minutes to pellet the cell debris.
- Transfer supernatant to a new tube for further analysis.