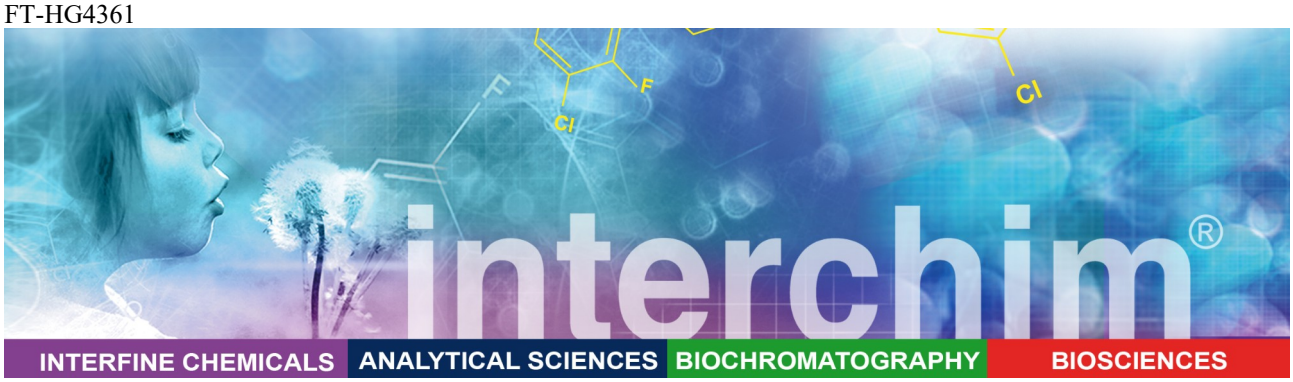
FT-HG4361



RIPA Lysis and Extraction Buffer

Product Description

Catalog #: HG4368, 50ml HG4361, 100ml HG4369, 500ml
Name: **RIPA Buffer**
Radio-Immunoprecipitation Assay
Storage: +4°C (L)

Introduction

The RIPA buffer is a reliable cell lysis buffer used to lyse cultured mammalian cells from both plated cells and cells pelleted from suspension cultures. RIPA Buffer enables efficient cell lysis and protein solubilization while avoiding protein degradation and interference with the proteins' immunoreactivity and biological activity.
RIPA buffer enables the extraction of cytoplasmic, membrane and nuclear proteins and is compatible with many applications, including reporter assays, protein assays, immunoassays and protein purification. It is furthermore compatible with the BC Assay Protein Assay #UP4084A. RIPA Buffer also results in low background in immunoprecipitation and molecular pull-down assays. Finally RIPA Buffer is widely used in many other applications to lyse mammalian cells.

Directions for use

• Our RIPA Buffer #HG4361 is ready-to-use and does not contain protease or phosphatase inhibitors. However, if desired, protease inhibitors, can be added to the RIPA buffer just before use to prevent proteolysis and maintain phosphorylation of proteins. Add protease and phosphatase inhibitors immediately before use.

• Use 1 ml of cold RIPA Buffer for every 5 × 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

Protocol [r]: Procedure for Lysis of Monolayer-cultured Mammalian Cells

Note: If desired, add protease and phosphatase inhibitors to the RIPA Buffer immediately before use
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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 cm2 flask containing 5 × 106 HeLa or A431 cells. Keep on ice for 5 minutes, swirling the plate occasionally for uniform spreading.

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 pellet 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 [r]: Procedure for Lysis of Suspension-cultured Mammalian Cells

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

1. Pellet the cells by centrifugation at 2,500 × g for 5 minutes. Discard the supernatant.

2. Wash cells twice in cold PBS. Pellet cells by centrifugation at 2,500 × g for 5 minutes.

3. Add RIPA Buffer to the cell pellet. Use 1 ml of RIPA buffer for 40 mg (~5 × 106 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.

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.

Troubleshooting

Problem	Possible Cause	Solution
Low total protein yield	Some cells are more resistant to lysis than others	Make sure the cell pellet is thoroughly suspended in RIPA Buffer and incubate for longer with occasional swirling − sonicate the pellet to increase yield
Low concentration of proteins	Excess buffer used	Use less buffer (for example, 0.25-0.5 ml per 75 cm2 flask containing 5 × 106 cells) − use a sufficient amount to cover the entire plate
Proteolysis	No protease inhibitors added	Add Protease Inhibitors to the buffer before use Phosphatase activity Add Halt™ Phosphatase I
Low phosphorylation of proteins	Phosphatase activity	Add Phosphatase Inhibitor #BT3250 to the buffer before use
	Protein is non-phosphorylated or poorly phosphorylated	None

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Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.
Protein Extraction Buffer #BZ2171
Detergents, Protease Inhibitors
Phosphate Buffered Saline, #UP68723A(pack of 10L) or #N13520(sterile, 1X)
Protein analysis: BC Assay Protein Assay #UP4084A, see electrophoresis product line
Protein purification

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06