RIPA Lysis Buffer

Cat. No.	size
E0295-01	50 ml
E0295-02	200 ml

Storage condition: Store at 4°C.

Contents: 25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40 substitute, 1% sodium deoxycholate, 0,1% SDS.

Important Product Information:

RIPA Buffer does not contain protease or phosphatase inhibitors. If necessery use protease and phosphatase inhibitors immediately before use.

Some protein kinases and other enzymes may be sensitive to the components of the RIPA Buffer, resulting in their decreased activity. In such cases, prepare a RIPA buffer that does not contain sodium deoxycholate and SDS.

RIPA Buffer (Radioimmunoprecipitation Assay) is a complete lysis solution reagent used for rapid and efficient total lysis and solubilization of protein from both adherent and suspension cultured mammalian cells, effectively extracting cytoplasmic, nuclear and membrane proteins.

Procedure for Lysis of Monolayer-cultured Adherent Mammalian Cells

- Carefully remove culture medium from adherent cells.
- Wash cells twice with chilled PBS.
- Add cold RIPA Buffer to the cells (1mL of buffer per 5 × 10^6 HeLa or A431 cells). Incubate on ice for 5 minutes, swirling the plate occasionally.
- Collect the lysate and centrifuge at ~14,000 × g for 15 minutes.
- Transfer supernatant to a new tube for further analysis.

Procedure for Lysis of Suspension-cultured Mammalian Cells

- Pellet the cells by centrifugation at 2500 × g for 5 minutes. Carefully remove and discard the supernatant.
- Wash cells twice in chilled PBS. Centrifuge at 2500 × g for 5 minutes. Carefully remove and discard the supernatant.
- Add chilled RIPA Buffer to the cell pellet (1mL of RIPA buffer for 5 × 10^6 of HeLa cells). Pipette the mixture up and down to suspend the pellet.
- Shake mixture gently for 15 minutes on ice. Centrifuge samples at 14,000 × g for 15 minutes.
- Transfer supernatant to a new tube for further analysis.

Procedure for Lysis of Tissues

- Place the fresh tissue into chilled PBS and rinse several times. Mince the tissue into small pieces.
- Add RIPA Buffer to the tissue at 10:1 (10mL chilled RIPA Buffer per gram of tissue).
- Homogenize for several minutes at high speed until no tissue chunks remain.
- Incubate on ice for 3 minutes.
- Centrifuge at 10000 x g for 10 minutes
- 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 pellet is throughly suspended in RIPA Buffer and incubate for longer with occasionaly swirling– sonicate the pellet to increase yield
Low concentration of protein	Excess buffer used	Use less buffer
Proteolysis	No protease inhibitors	Add protease inhibitor to the buffer before use
Low phosphorylation of	Phosphatase activity	Add phosphatase inhibitor to the buffer before use

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