PRODUCT INFORMATION

RIPA Buffer — Cat. no. 39244

Product Description:

General	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.
Important	The buffer does not contain any protease or phosphatase inhibitors. We would recommend: • Cat. no. 39102 Protease Inhibitor Mix M (for mammalian cells and tissues) • Cat. no. 39050 Phosphatase Inhibitor Mix I, powder • Cat. no. 39055 Phosphatase Inhibitor Mix II, solution

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

Use 1 ml of cold RIPA Buffer for every 5 x 10^6 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.

Lysis of monolayer-cultured mammalian cells

- Carefully remove the culture medium from the adherent cells.
- Wash cells twice with cold PBS.
- Add cold RIPA Buffer to the cells.
  Use 1 ml buffer per 75 cm^2 flask containing 5 x 10^6 HeLa or A431 cells.
  Keep on ice for 5 min, 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 x g for 15 min to pellet the cell debris.
  Note: To increase yields, sonicate the pellet for 30 seconds with 50 % pulse.
- Transfer the supernatant to a new tube for further analysis.

Lysis of suspension-cultured mammalian cells

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

Troubleshooting

Problem	Possible cause	Remedy
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 protein concentration	Excess buffer used	Use less buffer, e.g. 0.25 - 0.5 ml per 75 cm^2 flask containing 5 x 10^6 cells − Use sufficient amount to cover the entire plate
Proteolysis	No protease inhibitors added	Add Protease Inhibitor Mix M to the buffer before use
Low protein phosphorylation	Phosphatase activity	Add Phosphatase Inhibitor Mix I or II to the buffer before use
	Protein is non-phosphorylated or poorly phosphorylated	None

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