1X RIPA Lysis Buffer

Product No.: RIPA1

Package: 500ml

Storage/Stability: Store at 4°C upon receipt.

Use limitations:

For research use only. Not for therapeutic or diagnostic use.

Composition:

50mM Tris-HCl, 150mM Sodium chloride, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% SDS, 5mM EDTA

Note: *RIPA Lysis Buffer does not contain any protease inhibitors or phosphatase inhibitors.*
If desired, add inhibitors to RIPA Lysis Buffer immediately before applying to cells.

Procedure:

Note: *Perform all steps on ice.*

- **Lysis of adherent cultured cells**

1. Carefully remove (decant or aspirate) culture media from adherent cells.
2. Carefully wash cells twice with a volume of cold PBS equal to that of the culture media removed.
3. Add cold RIPA Lysis Buffer to the cells. Use 1 mLof RIPA Lysis Buffer for up to 5 x 106 cells.
4. Incubate on ice 15 minutes, occasionally swirling the plate to keep the surface evenly covered.
5. Use a cell scraper to remove lysate from the culture dish. Pass the cell lysate through a pipette several times to form a homogenous lysate.
6. Transfer lysate to a cold 1.5 mL microcentrifuge tube on ice.
7. Centrifuge the lysate at 14,000 x g for 15 minutes at 4°C to separate the total protein (supernatant) from the cellular debris (pellet).
8. Transfer the supernatant to a new 1.5 mL microcentrifuge tube on ice.
9. Total protein can be used immediately or stored frozen at -20°C until needed. Prepare small aliquots to avoid repeated freeze-thaw cycles, which may degrade the sample.

- **Lysis of suspension cultured cells**

1. Pellet cells by centrifugation at 2,500 x g for 5 minutes at 4°C. Discard the supernatant.
2. Wash the cell pellet twice in cold PBS using a volume equal to the culture media removed in step 1. Pellet the cells by centrifugation at 2,500 x g for 5 minutes at 4°C.
3. To the cell pellet obtained in step 3, add 1 mL of cold RIPA Cell Lysis Buffer for up to 5 x 106 cells. Pipette the mixture up and down to resuspend the pellet.
4. Shake the mixture gently for 15 minutes on ice.
5. Centrifuge the mixture at 14,000 x g for 15 minutes at 4°C to pellet the cell debris.

6. Transfer the supernatant containing total protein to a new cold 1.5 mL microcentrifuge tube.
7. Total protein can be used immediately or stored frozen at -20°C until needed. Prepare small aliquots to avoid repeated freeze-thaw cycles, which may degrade the sample.

Troubleshooting

Problem	Possible reason	Solution
Low total protein yield	Some cells are more resistant to lysis than others or cells were not completely lysed	Make sure the cell pellet is thoroughly resuspended in RIPA Lysis Buffer and increase incubation time on ice. Brief sonication at 50% plus may also increase yield.
Low concentration of proteins	Too few cells per volume of RIPA Cell Buffer were used	Increase number of cells or decrease volume of RIPA Lysis Buffer.
Proteolysis	No protease inhibitors added	Add Protease Inhibitor Cocktail to the buffer before use
Low phosphorylation of proteins	Phosphatase activity	Add Phosphatase Inhibitor Cocktail to the buffer before use

Related Products

#2040 Protease Inhibitor Cocktail I, for general use
#2041 Protease Inhibitor Cocktail II, animal free
#2042 Protease Inhibitor Cocktail I, for bacterial cell
#2043 Protease Inhibitor Cocktail III, for mammalian cell and tissue
#2046 Protease Inhibitor Cocktail IV, for fungal and yeast
#2047 Protease Inhibitor Cocktail V, EDTA free
#2049 Protease Inhibitor Cocktail VI, for general use
#2050 Protease Inhibitor Cocktail VI, plant cells
#ICT002 CyICT Protease + Phosphatase Inhibitor Cocktail TURBO