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MCE RIPA Lysis Buffer (Strong)

1 Contents

Cat. No.	Product Name	Package
HY-K1001-100 mL	RIPA Lysis Buffer (Strong)	100 mL

2 General Information

MCE RIPA (Radio-Immunoprecipitation Assay) Lysis Buffer (Strong) is one of the most reliable buffers used to lyse cells from both cultured cells and tissues. It is a widely used lysis and wash buffer for reporter assays, protein kinase assays, immunoassays and protein purification.

RIPA Lysis Buffer (Strong) consists of 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton x-100, 1% sodium deoxycholate, 0.1% SDS, and general protease and phosphatase inhibitors (e.g., sodium orthovanadate, sodium fluoride, EDTA). If desired, add MCE Protease Inhibitor (HY-K0010) and MCE Phosphatase Inhibitor Cocktails (e.g., HY-K0021, HY-K0022, and HY-K0023).

3 Protocol

Procedure for Cultured Cells

1. Pipette proper volume of RIPA Lysis Buffer and mix thoroughly. Add Protease Inhibitor Cocktail to lysis buffer to prevent proteolysis. Add Phosphatase Inhibitor Cocktails to maintain phosphorylation status of proteins as needed. Incubate on ice for subsequent use.

2. For adherent cells

a. Carefully remove culture medium from adherent cells.

b. Wash cells twice with cold wash solution, such as PBS, normal saline or serum-free culture medium, to remove residual medium.

c. Add proper volume of cold RIPA Lysis Buffer, then stroke with pipette until the buffer immerses cells completely. Incubate on ice and shake slightly for 5-10 minutes.

Note: Generally, use 1 mL of RIPA Lysis Buffer for 0.5-5 × 10^6 cells. The volume of added RIPA Lysis Buffer can be adjusted proportionally.

For suspension cultured cells

a. Collect cells into a centrifuge tube. Centrifuge samples at 500 g for 5 minutes and discard the supernatants.

b. Wash cells twice with cold wash solution, such as PBS, normal saline or serum-free culture medium, to remove residual medium.

c. Add proper volume of cold RIPA Lysis Buffer, then stroke with pipette until the buffer immerses cells completely. Incubate on ice and shake slightly for 5-10 minutes.

Note: Generally, use 1 mL of RIPA Lysis Buffer for 0.5-5 × 10^6 cells. The volume of added RIPA Lysis Buffer can be adjusted proportionally.

3. After lysis, centrifuge at 14,000 g for 5 minutes at 4 °C. Transfer the supernatants to a new tube for further analysis. Aliquot and freeze in liquid nitrogen and stored at –80°C for future use. Avoid multiple freeze/thaw cycles.

Procedure for Tissue Lysis

1. Pipette proper volume of RIPA Lysis Buffer and mix thoroughly. Add Protease Inhibitor Cocktail to lysis buffer to prevent proteolysis. Add Phosphatase Inhibitor Cocktails to maintain phosphorylation status of proteins as needed. Incubate on ice for subsequent use.

2. Cut tissue sample into small pieces on ice quickly to minimize protein degradation.

3. Add 150-250 µL cold RIPA Lysis Buffer per 20 mg of tissue sample and homogenize using electric homogenizer. Add more Lysis buffer if tissue is not completely lysed.

4. Transfer complete homogenized sample into a centrifuge tube. Incubate on ice and shake slightly for 5-10 minutes.

5. After lysis, centrifuge at 14,000 g for 5 minutes at 4 °C. Transfer the supernatants to a new tube for further analysis. Aliquot and freeze in liquid nitrogen and stored at –80°C for future use. Avoid multiple freeze/thaw cycles.

Note: 20 mg of frozen mouse liver tissues may yield 15-25 mg/mL protein.

4 Storage condition

Store at -20°C 12 months

5 Precautions

1. Protease Inhibitor Cocktail is not included in the MCE RIPA Lysis Buffer kit.
2. All steps should be performed either on ice or at 4°C.

3. RIPA Lysis Buffer contains ionic detergents and may not be suitable for some kinase enzyme assays.

4. Do not add phosphatase inhibitors when preparing lysates for phosphatase assays.

5. There might be some transparent gel complex containing genomic DNA in lysed proteins.

6. Use BCA Protein Assay kit (HY-K0401) to quantify lysed proteins. Bradford Protein Assay kit is not recommended.

7. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.