TargetMol®
A DRUG SCREENING EXPERT

Catalog No.C0045

RIPA Lysis Buffer (Strong)

(100 mL)

Instruction Manual

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Description

Targetmol RIPA Lysis Buffer is a traditional rapid lysis buffer for cellular tissues. RIPA stands for Radio Immunoprecipitation Assay. There are many different formulations of RIPA lysis buffer, which can be broadly categorized into strong, medium, and weak depending on the strength of the lysate. The protein samples obtained from the buffer can be used for general PAGE, Western Blot (WB), Immunoprecipitation (IP), Co-immunoprecipitation (Co-IP) and ELISA. This product can be used for cell or tissue samples of animals and plants, as well as fungal or bacterial samples.

The main components of a strong RIPA lysis buffer include 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin, and many other inhibitors. However, our RIPA lysis buffer does not contain all of the components of a protease/phosphatase inhibitor cocktail. To effectively prevent protein degradation, adding PMSF or Protease Inhibitor Cocktail (Cat. No. C0001) and Phosphatase Inhibitor Cocktail (Cat. No. C0002, C0003, C0004) is recommended.

Operation Manual

1. Lysing cell or bacterial yeast samples

Thaw the RIPA lysis buffer and mix well. Take an appropriate amount of buffer and add PMSF within minutes before use to give a final concentration of 1 mM PMSF, or add an appropriate amount of protease-phosphatase inhibitor cocktails as needed for the experiment. Operate on ice.

1) For adherent cells: remove the culture medium and wash twice with PBS, saline or serum-free medium (Skip the washing if the extracted protein is unaffected by self-contained proteins in the serum). Add RIPA buffer 150-250 μL per well on a 6-well plate. Gently aspirating and dispensing the liquid using a pipette to ensure thorough mixing. Leave on ice for 5-10 minutes, meanwhile, swirl the plate 3-4 times for 30 seconds each time to lyse the cells fully.

2) For suspended cells: collect cells by centrifugation and gently vortex or flick the bottom of the tube to disperse the cells as much as possible. Add 150-250 μL of lysate to each well of a 6-well plate and flick the bottom of the tube to fully lyse cells. There should be no obvious cell precipitation after full lysis. If the amount of cell is sufficient, it is necessary to reallocate the cells into 0.5-5 × 10^6 cells/tube before lysis. Leave on ice for 5-10 minutes, during which time it may be shaken 3-4 times for 30

seconds each time to lyse the cells fully.

3) For bacteria or yeast: for 1 mL of bacteria or yeast solution, centrifuge to remove the supernatant. Wash once with PBS if necessary, and gently vortex or flick the bottom of the tube to dislodge as much bacteria or yeast as possible after sufficiently removing the liquid. Add 100-200 μL of lysis buffer, gently vortex or flick the bottom of the tube to mix well, and lyse on ice for 2-10 minutes. If you want to get better lysis effect, bacteria and yeast can be digested with lysozyme and cell wall-degrading enzyme respectively, before lysing with the lysis buffer.

4) After sufficient lysis, centrifuge 10,000-14,000 g for 3-5 minutes, take the supernatant, and then proceed to the subsequent PAGE, Western and IP operations, or use liquid nitrogen to freeze and store under -80°C for long-term storage.

2. Lysing tissue samples

1) Mince the tissue into small pieces.

2) Melt the RIPA lysis buffer and mix well. Take an appropriate amount of buffer and add PMSF within minutes before use to make a final concentration of 1 mM PMSF, or add an appropriate amount of protease-phosphatase inhibitor cocktails as needed for the experiment.

3) Add RIPA Lysis Buffer to the tissue at the ratio of 150-250 μL lysis buffer per mg of tissue. (For incompletely lysed tissues, the volume of RIPA Lysis Buffer can be increased. To obtain samples with high protein concentration, the volume of RIPA Lysis Buffer can be reduced.)

4) Homogenize on ice with a glass homogenizer 30-50 times or sonicate the tissue for 30 seconds 3-4 times at 1 minute intervals on ice for cooling. Then inspect the sample with a microscope. The cell rupture rate should be no less than 90%, and the tissue should be completely lysed without obvious fragments. Transfer the homogenate to a tube and keep on ice for 5-10 minutes, shaking it 3-4 times for 30 seconds each time to ensure thorough lysis.

5) After sufficient lysis, centrifuge 10,000-14,000 g for 3-5 minutes, take the supernatant, and then proceed to the subsequent PAGE, Western and IP operations. The supernatant obtained after every 20 mg of frozen mouse liver tissue with 200 μL of this lysate may yield 15-25 mg/mL protein. Protein extraction varies for different tissues in different states.

Note: It is normal to see some transparent gel-like complex containing genomic DNA in the lysate of RIPA lysis buffer. In the case of not detecting proteins that are tightly bound to genomic DNA, the

supernatant can be used for further analysis after centrifugation. Otherwise, sonicate the gel-like complex and then centrifuge to collect supernatant for further experiments. Common transcription factors such as NF-kappa B and p53 can be detected without sonication.

Precautions

1) Avoid multiple freeze/thaw cycles. It is recommended to aliquot the samples for future use.

2) This product contains some inhibitors that partially inhibit protein degradation, but does not contain PMSF or protease-phosphatase inhibitor cocktails.

3) Add tissues directly to the lysis buffer after appropriate shearing If the tissue itself is very small, and thoroughly lyse the samples by vortexing vigorously. Then centrifuge and collect the supernatant for further experiments. Direct lysis is more convenient, as it eliminates the need for homogenizers or grinding equipment, but it may not achieve as thorough lysis as homogenization or grinding.

4) All steps should be performed on ice.

5) The SDS in the lysis buffer may precipitate at 4°C. Completely redissolve the buffer by heating in a 37°C water bath to return to room temperature before use.

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

7) Do not add phosphatase inhibitors when preparing lysates for phosphatase assays.

8) RIPA lysis buffer extracts all proteins, including nuclear proteins, cytoplasmic proteins, membrane proteins, etc. If specific proteins (e.g., nuclear proteins and membrane proteins) need to be extracted, specialized protein extraction kits should be used.

9) If the target protein cannot be precipitated during immunoprecipitation, it indicates that the lysis buffer is too strong and a gentle lysis buffer such as C0047 RIPA Lysis Buffer (Weak) is recommended.

10) This product is for R&D use only, not for diagnostic procedures, food, drug, household, or other uses.

11) Please wear a lab coat and disposable gloves.

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Storage Condition

Store at -20°C for 12 months.

Comparison between 3 lysis buffers

Product Name	RIPA Lysis Buffer (strong)	RIPA Lysis Buffer (medium)	RIPA Lysis Buffer (weak)
Product Number	C0045	C0046	C0047
Effective Lysis Components	1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS	1% NP-40, 0.5% deoxycholate, 0.1% SDS	1% NP-40, 0.25% deoxycholate
Lysis Strength	Strong	Medium	Weak
Efficacy of Membrane Protein Extraction	Excellent	Good	Moderate
Efficacy of Cytoplasmic Protein Extraction	Excellent	Excellent	Excellent
Efficacy of Nuclear Protein Extraction	Excellent	Good	Good
Efficacy of Cytoplasmic Phosphoprotein Extraction	Good	Good	Good
Efficacy of Nuclear Transcription Factor Extraction	Good	Good	Good
Containing Protease Inhibitor	Yes	Yes	Yes
Containing Phosphatase Inhibitor	Yes	Yes	Yes
Sample compatibility of different species	Yes	Yes	Yes
Primary Purpose	WB, IP	WB, IP	WB, IP, co-IP

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