PROMPT
Proteins for Research

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RIPA Buffer (Strong)

G2002

Introduction

RIPA Lysis Buffer is a traditional rapid lysis solution for cells and tissues. It is available in a variety of formulations and classified as strong, medium or weak. The protein samples obtained from the lysis of tissues and cells in RIPA strong lysis solutions can be used for routine PAGE, Western and other experiments where protein activity is not strictly required. This product contains 50mM Tris-HCl (pH 7.4), 150mM NaCl, 1mM EDTA-2Na, 1% Triton X-100, 1% sodium deoxycholate and 0.1% SDS. It is suitable for animal or plant tissues and cells, but can also be used for fungal or bacterial samples.

Size

1×100mL

Form

Colorless liquid

Storage

Ship with wet ice; Store in the dark at 2-8℃; valid for 18 months.

Protocol

Requires self-provide protease inhibitors. Protease inhibitors should be added to RIPA Lysis (Strong) before use to prevent protein degradation. All references to RIPA Lysate (Strong) in the following instructions have been added with protease inhibitors.

- **For tissue samples:**

1. The tissue pieces are washed with pre-cooled PBS to remove blood stains, cut into small pieces and placed in the homogenizer.
2. Add 10 times the tissue volume of RIPA Lysis Buffer (Weak) and homogenate at low temperature. Note that the amount of RIPA Lysis Buffer (Weak) can be added in a ratio of approximately 50 mg of tissue to 1mL of lysate. If the content of tissue protein is low, the amount of lysate can be reduced to increase the protein concentration in crude extract solution.
3. Transfer the homogenate to a 1.5mL of centrifuge tube and oscillate. Ice bath for 30 minute with repeated pipetting every 10 minute to ensure the complete lysis of tissue cells.
4. Centrifuge at 12000 x g for 5 minute and collect the supernatant as total protein solution.

- **For adherent cells:**

1. Wash the cells with PBS for 2-3 times and aspirate the residual liquid thoroughly at the last time.
2. Aspirate RIPA Lysis Buffer (Weak) into the cell culture plate and flask at a ratio of 250μL of cell lysate per well of 6-well plate, shake the culture plate and flask repeatedly to make the lysate fully contact with cells for 3-5 minute.
3. Scrape off the cells with a cell scraper and collect into a centrifuge tube.
4. Lysis on ice for 30 minute.
5. Centrifuge at 12000 x g for 5 minute and collect the supernatant as total protein solution.

- **For suspension cell samples:**

1. Centrifuge to collect cells.
2. Mix the cytosol with RIPA Lysis Buffer (Weak) at a ratio of 250μL of lysate per cell well of a 6-well plate and shake.
3. Ice bath for 30 minute, repeatedly pipetting every 10 minute to ensure complete cell lysis.
4. Centrifuge at 12000 x g for 5 minute and collect the supernatant as total protein solution.

- **For bacterial or fungal samples:**

1. Remove 1mL of the suspension, centrifuge to remove the supernatant and wash once with PBS to fully remove the liquid. Vortex to disperse the bacterium as much as possible.
2. Add 100-200μL of RIPA Lysis Buffer (Strong) and vortex gently to mix well with the lysate.
3. Ice bath for 30 minute, repeatedly pipetting every 2 minute to ensure complete cell lysis.
4. Centrifuge at 12000 x g for 5 minute and collect the supernatant as total protein solution

Note

1. Tissue or cells may appear viscous when lysed. This can be achieved by repeatedly blowing with a pipettor or shaking with a vortexer until it is liquid. Add a further appropriate amount of lysis solution if it remains thick.
2. This reagent does not contain protease inhibitors. Add your own protease inhibitors before use.
3. For your safety and health, please wear safety glasses, gloves, or protective clothing.