

RIPA buffer(high efficiency)

Cat number: R0010

Size: 20ml/100ml, included PMSF (0.3ml/1.5ml).

Storage: RIPA stored at 4°C , PMSF stored at -20°C .

Introduction

The RIPA buffer is one of the most reliable buffers used to lysis cultured mammalian cells from both plated cells and cells pellet 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.

Protocol

If have precipitation, dissolve in 20°C water bath. Working solution: 1ml RIPA add 10ul PMSF to final concentration 1mM, mix thoroughly. According application prepare lysate buffer properly. Sample lysis should placed on ice or operate at low temperature situation for 20-30 min.

I. Sample Preparation

1. For adherent cells: Remove culture medium from adherent cells, wash with PBS, saline or serum-free medium. For 6 well plate, each well add 150~250ul lysate buffer, mix thoroughly. Pipette the mixture up and down to suspend the pellet.
 2. For suspension cells: Centrifuge cells, flip tube to separate cells. For 6 well plate, each well add 150~250ul lysate buffer, mix thoroughly. After fully lysis there should no obvious precipitation. For large cell amount, split cells into 50-1 million cells / tube.
 3. For tissue sample: Cut tissue to debris, 20mg sample add 150~250ul lysate buffer. Add lysate buffer properly to fully lysis cells. To obtain concentrated protein extracts, reduce lysate buffer properly. Homogenize using homogenizer.
- II. Centrifuge for 3-5 min at 10000~14000g, take the above supernate for protein concentration detection, SDS-PAGE, Western blotting and Immunoprecipitation, etc.

Note

- 1) RIPA buffer has high efficiency lysis ability, it can extract nucleoprotein, but at the same time the genome will be released. High cell density results in relatively ropiness, then ultrasound or boiling treatment is need.
- 2) RIPA Buffer is compatible with the Solarbio BCA Protein Assay Kit (Product No PC0020) and Lowry Protein Assay Kit (Product No PC0030)
- 3) PMSF should be added before use, protease inhibitor mixture(Product No P6730) and phosphatase inhibitors mixture(Product No P1260) can cooperate with the RIPA buffer.