MQ RIPA Buffer

Cat No. RIP-M-001-100
Size: 100ml, included PMSF (0.5ml)
RIPA stored at 4°C , PMSF stored at -20°C

MOLEQULE-ON®

Description

The MQ 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 precipitation occur, dissolve in 20°C water bath.

Working solution: Mix 1ml RAPA and 10µl PMSF to make final concentration of 1mM, mix thoroughly. Lysis of samples should be performed on ice or at low temperature 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, add 150~250µl lysate buffer in each well, mix thoroughly. Pipette the mixture up and down to suspend the pellet.
2. For suspension cells: Centrifuge cells, tilt tube to separate cells. For 6 well plate, add 150~250µl lysate buffer in each well, mix thoroughly. After fully lysis, there should be no obvious precipitation. For large cell amount, split cells into 50-1 million cells / tube.
3. For tissue sample: Cut tissue to debris, add 150~250µl lysate buffer in 20mg sample. 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 supernatant for protein concentration detection, SDS-PAGE, Western blotting and Immuno-precipitation, etc.

Notes:

MQ 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 ultrasounic bath or boiling treatment is need.
RIPA Buffer is compatible with the MOLEQULE-ON BCA Protein Assay Kit and Lowry Protein Assay Kit.
PMSF should be added before use, protease inhibitor mixture and phosphatase inhibitors mixture can cooperate with the RIPA buffer.