RIPA Buffer

Catalogue Number: MP015

Description: 150mM Sodium chloride, 50mM Tris-HCl (pH 7.5), 1% triton X-100,1% sodium deoxycholate, 0.1% SDS, and 2mM EDTA. The RIPA buffer from M&C Gene Technology is a reliable cell lysis buffer used to lyse cultured mammalian cells from both cells growing as monolayer and cells growing in suspension, and variety of tissues. It enables the quick extraction of membrane, cytoplasmic, and nuclear proteins and buffer components are compatible with many applications, including gene reporter assay, protein activity assay, immuno-assay, and protein purification. RIPA Buffer from M&C does not contain protease or phosphatase inhibitors. Protease and phosphatase inhibitors need to be added to the RIPA buffer just before use to prevent proteolysis and maintain phosphorylation of proteins.

Packing Size: 100 ml

Storage: 4°C

Application: For whole cell lysate preparation.

Preparation of Cell Lysates: **Add proteinase and phosphatase inhibitors as desired and DTT (1mM) before use.**

Cell culture device	24-well	12-well	6-well	35-mm	60-mm	100-mm	150-mm
Volume of RIPA	200 μL	200 μL	300 μL	300 μL	500 μL	1 mL	1 mL

1. For cell culture devices including 24-well, 12-well, 6-well and 35-mm plates
   - Remove the media from the tissue culture plate wells.
   - Wash cells twice with ice-cold PBS or TBS buffer.
   - Add RIPA Buffer (see above table for the amount) into the well to cover the cells.
   - Incubate the plate on shaker platform at 4 ºC for 30 minutes.
   - Transfer all contents including RIPA buffer and cell debris into 1.5-mL eppendorf tube.
   - Pellet cells at maximum speed for 10 minutes in the refrigerated microcentrifuge to pellet cell debris.
   - Harvest supernatants into fresh tubes.
   - Check protein concentration.
2. For cell culture devices including 60-mm, 100-mm, and 150-mm plates or flasks with similar size of culture surface
   - Remove the media from the tissue culture plate.
   - Wash cells in the plate or flask with 10 mL ice-cold PBS or TBS, repeat this two more times.
   - Harvest cells with 10 mL PBS or TBS in 15-mL tubes.
   - Pellet cells at 500xg for 10 minutes in the refrigerated microcentrifuge.
   - Resuspend cells in 1 mL RIPA buffer and transfer the cells into 1.5-mL eppendorf tube.
   - Incubate cells on ice for 30 minutes.
   - Spin cells at maximum speed for 10 minutes in the refrigerated microcentrifuge to pellet cell debris.
   - Harvest supernatants into fresh tubes.
   - Check protein concentration.