RIPA Buffer, Modified

Catalogue #: MP015M
Storage: 4 ºC
Size: 100mL

Description:

150mM Sodium chloride, 50mM Tris-HCl (pH 7.5), 0.1% Tween 20, 1mM EDTA, 1mM EGTA. The modified RIPA buffer from M&C Gene Technology is a reliable cell lysis buffer with gentle condition 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 and gentle 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. In particular, the modified RIPA Buffer from M&C provides an optimal condition for protein-protein association in immunoprecipitation application. The modified RIPA 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.

Application:

For whole cell lysate preparation with gental condition.

Preparation of Cell Lysates:

Add proper proteinase and phosphatase inhibitors as desired and DTT (final concentration: 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 the 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.
- Spin the 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 the cells with 10 mL ice-cold PBS or TBS, repeat this two more times.
- Harvest the cells with 10 mL PBS or TBS in 15-mL tubes.
- Pellet the cells by spinning cells at 500xg for 10 minutes in the refrigerated microcentrifuge.
- Resuspend the cells in 1 mL RIPA buffer and transfer the cells into 1.5-mL eppendorf tube.
- Incubate the cells on ice for 30 minutes.
- Spin the cells at maximum speed for 10 minutes in the refrigerated microcentrifuge to pellet cell debris.
- Harvest supernatants into fresh tubes.
- Check protein concentration.