10x RIPA Lysis Buffer

Cat#: orb1109247 (Manual)

General Information

10x RIPA Lysis Buffer is formulated for efficient and complete cell lysis and Solubilization of proteins. 10x RIPA Lysis Buffer enables protein extraction from cytoplasmic, membrane and nuclear proteins and is compatible with several applications including western blotting and immunoprecipitation.

Protocol for Cell Lysis

1. Resuspend and wash 1x106 cells in chilled PBS and discard the supernatant.
2. Dilute 10x RIPA Lysis Buffer to a 1x solution using ddH2O.
Addition of PMSF to cold 1x RIPA Lysis Buffer may result in formation of precipitates.
3. Lyse cell pellet in 0.1 ml 1x RIPA Lysis Buffer and vortex thoroughly. To increase yield, snap freeze and thaw samples 2-3 times until all the cells burst.
Note that 10x RIPA Lysis Buffer does not include protease or phosphatase inhibitors. If desired, protease and phosphatase inhibitors can be added to the RIPA buffer just prior to use to prevent proteolysis and maintain phosphorylation status of proteins.
4. Keep samples in ice for 10-15 minutes to dissolve structures and complexes.
5. Centrifuge at 4°C, 13.000 rpm for 10 minutes
6. Take the supernatant in a new eppendorf tube, quantify, add Laemmli Sample Buffer, boil for 5 minutes.

Protocol for Tissue Lysis

1. Dilute 10x RIPA Lysis Buffer to a 1x solution using ddH2O.
Addition of PMSF to cold 1x RIPA Lysis Buffer may result in formation of precipitates.
Note that 10x RIPA Lysis Buffer does not include protease or phosphatase inhibitors. If desired, protease and phosphatase inhibitors can be added to the RIPA buffer just prior to use to prevent proteolysis and maintain phosphorylation status of proteins.
2a. Cut the tissue sample and determine the amount of tissue by weighing. Grind the sample in a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw. Immediately, add 500 µl 1x RIPA Lysis Buffer to grinded tissue. Transfer the grinded and homogenized tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Mix well by vortexing for 10-15 seconds.

2b. Cut the tissue sample and determine the amount of tissue by weighing. Transfer the tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Add 500 µl 1x RIPA Lysis Buffer. Immediately and vigorously homogenize using a conventional rotor-stator homogenizer with a stainless steel probe at 15,000 rpm for 30 seconds.

3. To increase yield, snap freeze and thaw samples 2-3 times until all the cells burst. Keep samples in ice for 10-15 minutes to dissolve structures and complexes.

4. Centrifuge at 4°C, 13.000 rpm for 10 minutes.

5. Take the supernatant in a new eppendorf tube, quantify, add Laemmli Sample Buffer, and boil for 5 minutes.