T-Pro RIPA Lysis Buffer (1X)

Store at 2-8 °C

(JT89-L001M) 500 ml

T-Pro Biotechnology

This product is for laboratory research ONLY and not for diagnostic use.

Introduction	T-Pro RIPA Lysis Buffer is ready-to-use as a working 1X solution and requires no further dilution. T-Pro RIPA Lysis Buffer is intended for the extraction of cellular proteins for the efficient lysis of cells and solubilization of protein, while minimizing protein degradation and maintaining protein immunoreactivity and biological activity. We recommend using 1.0 mL of RIPA Lysis Buffer to lyse 0.5 to 5 x 10^7 adherent mammalian cells. This buffer contains ionic detergents and may not be suitable for kinase enzymes, if these enzymes are easily denatured. Do not add phosphatase inhibitors when preparing lysates for phosphatase assays. 1X T-Pro RIPA lysis buffer consists of 50 mM Tris HCl, 150 mM NaCl, 1.0% (v/v) NP-40, 1% (w/v) Sodium Deoxycholate and 0.1% (w/v) SDS at a pH of 7.4. This buffer was meticulously prepared using ultrapure reagents dissolved in highly polished pharmaceutical grade deionized water. Protease and phosphatase inhibitors are recommended but not included in product composition. Recommended final concentrations of protease inhibitors: 1.0 mM Phenylmethylsulfonyl fluoride (PMSF), 10 µM Leupeptin, 0.1 µM Aprotinin, 1.0 µM Pepstatin. Recommended final concentrations of phosphatase inhibitors: 1.0 mM Na3VO4, 1.0 mM NaF.
Materials needed but not provided	1. Centrifuge 2. Microcentrifuge tubes 3. PBS wash buffer: 10 mM KH2PO4, 150 mM NaCl, pH 7.4 4. Protease Inhibitor Cocktails (if desired) 5. Phosphatase Inhibitor Cocktails (if desired)
Storage	T-Pro RIPA Lysis Buffer is stable for 2-8 °C

Instructions

A. Sample preparation

1 Adherent Cells
Remove growth medium from the cells by decantation or aspiration.
Carefully wash cells twice with a volume of ice cold PBS equal to that of the culture media removed.

2 Suspension Cells
Pellet the cells by centrifugation at 2,500 x g for 5 minutes. Discard the supernatant.
Carefully wash cells twice with a volume of ice cold PBS equal to that of the culture media removed.

B. Sample lysis

1 After removal of the final wash solution from the cells, add an appropriate volume of RIPA Buffer (1 mL for 0.5 to 5 x 10^7 cells). Incubate on ice or in a refrigerator (2-8 °C) for 5-15 minutes.

2 Use cell scraper to scrape off cells. Pass the cell lysate through pipette several times to form a homogeneous lysate and transfer lysate to a 1.5 mL microcentrifuge tube on ice.

3 Centrifuge the lysate at 14,000 x g for 15 minutes at 4 °C to pellet the cell debris.

4 Transfer supernatant to a new tube for further analysis.

5 If necessary, aliquot the protein samples for long-term storage at -20 °C. Repeated freeze and thaw cycles cause protein degradation and should be avoided.

* NOTE: If a mucoid aggregate of denatured nucleic acids is present, carefully remove it with a micropipette before centrifugation.