YamayBio

RIPA Lysis Buffer (Strong)

QUICK MANUAL

For research use only.
Not for use in diagnostic procedures.

Contents and Storage

RIPA Lysis Buffer (Strong)

BF7471 100 mL

Storage: Store at 4 °C for 12 months. The product is shipped at room temperature.

Introduction

RIPA Lysis Buffer is used for the efficient extraction of soluble proteins from animal cells and tissues. This buffer is compatible with a broad range of downstream applications, including Western Blotting, Immunoprecipitation, and Enzyme-Linked Immunosorbent Assays (ELISA). Protein samples prepared using RIPA Lysis buffer can be quantified using a BCA Protein Assay kit (Cat No.: BH5484).

Important Note:

1. The SDS in the RIPA lysis buffer may precipitate when stored at 4 °C. If precipitation is observed, warm the buffer to 37 °C and ensure it is completely dissolved before use. Once the buffer has returned to room temperature, it is ready for use.
2. All sample lysis steps should be performed on ice or at 4 °C to maintain protein integrity.
3. Use 100 µL of cold RIPA Lysis Buffer per 1 × 10^6 cells or 200 µL per 20 mg of tissue sample.
4. Add protease inhibitors (Cat. No.: BH5481) to the RIPA lysis buffer at a 1:100 (v/v) ratio within a few minutes before use.
5. Pellet Formation: It is normal to observe a small pellet in the final lysate product, consisting of genomic DNA entangled with proteins.

- If proteins tightly bound to genomic DNA are not of interest, centrifuge the lysate and use the resulting supernatant directly for subsequent experiments.
- If detection of these DNA-binding proteins is required, resuspend the pellet and disrupt it by sonication. Following sonication, centrifuge the lysate, and use the resulting supernatant for subsequent experiments.
- For the detection of common transcription factors, such as NF-κB and p53, experiments can typically be completed using the supernatant obtained after the initial centrifugation, without the need for sonication.

Quick Start Protocol

A. Lyse Adherent Cultured Mammalian Cells

1. Carefully decant the culture medium from adherent cells.
2. Wash the cells twice with cold PBS.
3. Remove PBS, then add cold RIPA lysis buffer to the cells (100 µL per 1 × 10^6 cells; for example, 100 µL per well of a 6-well plate). Gently pipette the cells and swirl the plate occasionally.
4. Scrape the lysate to one side, collect it, and transfer it to a microcentrifuge tube. Centrifuge at 12,000 × *g* for 15 minutes to pellet cell debris.
5. Transfer the supernatant to a new tube for further analysis. For long-term storage, store at -80 °C.

B. Lyse Suspension Mammalian Cells

1. Collect cells by centrifugation at 2500 × *g* for 5 minutes. Discard the supernatant.
2. Wash the cells twice with cold PBS. After each wash, collect cells by centrifugation at 2500 × *g* for 5 minutes.
3. Add cold RIPA Lysis Buffer to the cell pellet (100 µL per 1 × 10^6 cells). Gently pipette the pellet to resuspend.
4. Gently agitate the mixture on ice for 15 minutes.
   Centrifuge at 12,000 × *g* for 15 minutes.
5. Transfer the supernatant to a new tube for further analysis. For long-time storage, store at -80 °C.

C. Lyse Tissue Samples

1. Finely mince the tissue sample using surgical scissors.
2. Add cold RIPA lysis buffer to the tissue (200 µL per 20 mg of tissue).
3. Homogenize the sample thoroughly using a glass grinder until complete lysis is achieved. Add an additional 100-200 µL of RIPA Lysis Buffer if needed to ensure adequate lysis.
4. Centrifuge the lysate at 12,000 to 14,000 × *g* for 15 minutes.
5. Transfer the supernatant to a new tube for further analysis. For long-term storage, store at -80 °C.