YamayBio

RIPA Lysis Buffer (Strong)

QUICK START GUIDE

Research use only.
Not for diagnostic procedures.

Contents and storage

RIPA Lysis Buffer (Strong)

BF7471 100mL

Storage: Store at 4°C for 12 months. Product shipped at room temperature.

Introduction

RIPA Lysis Buffer is primarily used for the efficient extraction of soluble proteins from animal cells and tissues. This buffer is compatible with a wide range of downstream applications, including Western Blotting, Immunoprecipitation, and Enzyme-Linked Immunosorbent Assays (ELISA). Protein samples lysed using RIPA 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 it returns to room temperature, the buffer can be used.
2. All sample lysis steps are done on ice or at 4°C to maintain protein integrity.
3. Use 100 µL of cold RIPA Lysis Buffer for every 1 × 10^6 cells or 200 µL for every 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, which is a complex 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, the pellet can be resuspended and disrupted by sonication. Following sonication, centrifuge the lysate, and the supernatant can then be used for subsequent experiments.
- For the detection of some 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 cells twice with cold PBS.
3. Remove PBS, then add cold RIPA lysis buffer to the cells (100 µL per 1 × 10^6 cells; e.g., 100 µL per well of a 6-well plate). Gently pipette 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, package and store at -80°C.

B. Lyse suspension mammalian cells

1. Collect cells by centrifugation at 2500 × *g* for 5 minutes. Discard supernatant.
2. Wash cells twice with cold PBS. Collect cells by centrifugation at 2500 × *g* for 5 minutes after each wash.
3. Add cold RIPA Lysis Buffer to the cell pellet (100 µL per 1 × 10^6 cells). Gently pipet the pellet to resuspend.
4. Gently shake 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. Package and store at -80°C for long-term storage.

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 fully lysed. Add an additional 100-200 µL of RIPA Lysis Buffer if needed for adequate lysis.
4. Centrifuge the lysate at 12,000 - 14,000 × *g* for 15 minutes.
5. Transfer the supernatant to a new tube for further analysis. Package and store at -80°C for long-term storage.