RIPA Lysis Buffer (Medium)

R1505849

Storage -20℃. Store in the dark.

Introduction

Aladdin RIPA Lysis Buffer is a classical rapid lysis reagent for cells and tissues. Protein extracts prepared using this product are suitable for various downstream applications, including routine PAGE electrophoresis, Western blotting, immunoprecipitation (IP), co-immunoprecipitation (Co-IP), and ELISA.

This product is widely applicable to cell or tissue samples derived from animal and plant sources, and can also be used for fungal and bacterial samples.

The moderate-strength RIPA Lysis Buffer is composed of 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS. It is supplemented with protease and phosphatase inhibitors such as sodium fluoride and EDTA to effectively inhibit protein degradation and maintain sample integrity.

For protein concentration quantification of lysates obtained with this product, the BCA protein assay kit is recommended. It should be noted that due to the high concentration of detergents in the lysis buffer, the Bradford method is not suitable for determining the protein concentration of these samples.

Instructions for Use

Thaw the RIPA lysis buffer and mix thoroughly. Shortly before use, add an appropriate volume of PMSF (to a final concentration of 1 mM) or a protease/phosphatase inhibitor cocktail to a suitable amount of the lysis buffer.

1. For Lysis of Adherent Cells:

Remove the culture medium and wash the cells twice with PBS. Completely remove any residual liquid.Add 100–200 µl of lysis buffer containing inhibitors per 0.5–1 million cells (equivalent to one well of a 6-well plate). Gently pipet or swirl to ensure complete contact between the lysis buffer and cells.Lysis of animal cells typically occurs within 1–2 seconds of contact. For plant cells, lyse on ice for 2–10 minutes.

2. For Lysis and Preparation of Suspension Cells:

Collect the cells by centrifugation. Wash the pellet twice with PBS and completely remove the supernatant.Gently vortex or flick the tube to disperse the cell pellet.Add 100–200 µl of lysis buffer containing inhibitors per 0.5–1 million cells. Mix thoroughly.Incubate the mixture on ice for 5–20 minutes, mixing intermittently by gentle flicking or pipetting.After complete lysis, no significant cell pellet should remain.For larger cell quantities, aliquot into tubes containing 0.5–1 million cells per tube before lysis. Large clumps are difficult to lyse completely, while smaller numbers of cells lyse more efficiently due to better contact with the lysis buffer.

3. For Lysis and Preparation of Bacterial or Yeast Samples:

For 1 ml of bacterial or yeast culture, pellet the cells by centrifugation. Wash the pellet twice with PBS and completely remove the supernatant.Gently vortex or flick the tube to resuspend and disperse the cells.Add 100–200 µl of lysis buffer. Mix by gentle vortexing or flicking.Lyse the cells on ice for 2–10 minutes.For more efficient lysis, bacterial samples can be pretreated with lysozyme and yeast samples with lyticase prior to adding the lysis buffer containing inhibitors.

4. For Lysis and Preparation of Tissue Samples:

Mince the tissue into small fragments.Add approximately 100–200 µl of lysis buffer per 20 mg of tissue.If lysis is insufficient, increase the amount of lysis buffer accordingly. To obtain a more concentrated protein extract, reduce the volume of lysis buffer.Homogenize the mixture using a glass homogenizer or other suitable homogenization equipment. Thorough homogenization is essential for complete tissue lysis.Centrifuge the lysate at 12,000 × g for 5 minutes at 4°C. Collect the supernatant for subsequent protein concentration determination.

Precautions

1. To ensure optimal performance, minimize repeated freeze-thaw cycles. It is recommended to aliquot the product for use.
2. All sample lysis procedures should be performed on ice or at 4°C to minimize potential protein degradation.
3. This RIPA Lysis Buffer already contains protease and phosphatase inhibitors. For enhanced performance, consider supplementing with additional protease inhibitor cocktail (P301902) manufactured by Aladdin.
4. Lysates prepared with RIPA lysis buffer often contain a small, translucent gelatinous mass, which is a normal phenomenon. This substance consists of complexes formed by genomic DNA and other cellular components. If you are not detecting proteins that tightly bind to genomic DNA, you can simply centrifuge the lysate and use the supernatant for subsequent experiments. If detection of such proteins is required, the translucent mass can be dispersed by sonication, followed by centrifugation to collect the supernatant for downstream applications. However, for the detection of common transcription factors such as NF-kappaB and p53, sonication is generally not necessary.