RIPA Lysis Buffer (Strong)

Introduction

RIPA Lysis Buffer (Strong) is widely used for reporter gene assays, protein kinase experiments, immunoassays, and protein purification. Its core components are 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and common protease and phosphatase inhibitors (Sodium orthovanadate, Sodium fluoride, EDTA), which effectively prevent protein degradation. This lysis buffer does not contain a complete protease and phosphatase inhibitor cocktail. For optimal protein protection, please add a comprehensive protease and phosphatase inhibitor cocktail additionally.

This product is suitable for protein extraction from animal cells and tissues. The obtained protein samples can be used in a variety of routine immunological assays, such as Western blot (WB), immunoprecipitation (IP), and enzyme-linked immunosorbent assay (ELISA).

For 100 mL of this product, 150–250 μL of lysis buffer is required per well in a 6-well plate, allowing one bottle to be used for approximately 400–666 wells (samples). For tissue samples, 150–250 μL of lysis buffer is required per 20 mg of tissue, so one bottle can be used for approximately 400–666 extractions. The exact volume can be adjusted according to sample density and lysis efficiency.

Components and Storage

Size / Components	100 mL	Storage
RIPA Lysis Buffer (Strong)	100 mL	-20°C
Shiping：Blue ice	Shelf life: 12 months	

Protocol

1. Lysis of cell samples

(1) Preparation of lysis buffer

Dissolve and mix the RIPA Lysis Buffer (Strong), take an appropriate volume and keep it on ice. If needed, add Protease Inhibitor Cocktail (K1007), Phosphatase Inhibitor Cocktails (K1015), or Deacetylase Inhibitor Cocktail (K1017) shortly before use. Please refer to the corresponding product manuals for detailed instructions. After preparation, keep the buffer on ice.

(2) Cell lysis

- Adherent cells

  Remove the culture medium and wash the cells twice with pre-chilled PBS, saline, or serum-free medium (washing off serum IgG facilitates IP and Co-IP experiments but is not required for WB. If serum proteins do not interfere with subsequent assays, washing can be omitted). Add pre-chilled lysis buffer at 150–250 µL per well of a 6-well plate. Pipette up and down several times to ensure full contact between lysis buffer and cells. Incubate on ice for 5–10 min, vortexing vigorously 3–4 times for 30 s each to achieve complete lysis.

- Suspension cells

  Centrifuge to collect cells and discard the supernatant. Wash the cells twice with pre-chilled PBS, saline, or serum-free medium (removal of serum IgG helps IP and Co-IP, but is not required for WB; if serum proteins do not interfere, washing can be omitted). Add pre-chilled lysis buffer at 150–250 µL per 6-well equivalent. Gently tap the bottom of the tube with your finger to fully lyse the cells. After sufficient lysis, there should be no obvious cell pellet. If the cell number is high, aliquot into tubes containing 5–10 × 10^5 cells per tube before lysis. Incubate on ice for 5–10 min, vortexing vigorously 3–4 times for 30 s each to ensure complete lysis.

(3) After complete lysis, centrifuge at 14,000 × g at low temperature for 5 min. Collect the supernatant for subsequent WB, IP, PAGE, etc. Alternatively, snap-freeze the lysate in liquid nitrogen and store at -80 °C for long-term use.

***Note:** In general, 150 µL lysis buffer per well of a 6-well plate is sufficient. For very high cell density, increase to 200–250 µL. Add 100 µL lysis buffer per 1 × 10^6 mammalian cells; the protein concentration of the resulting supernatant is typically about 2–4 mg/mL (varies by cell type).

2. Lysis of tissue samples

(1) Dissolve and mix the RIPA Lysis Buffer (Strong), take an appropriate volume and place it on ice. If needed, add Protease Inhibitor Cocktail (K1007), Phosphatase Inhibitor Cocktails (K1015), or Deacetylase Inhibitor Cocktail (K1017) a few minutes before use, following the respective product manuals. Keep the prepared buffer on ice.

(2) Place the tissue in a small dish on ice and mince it into fine pieces.

(3) Add lysis buffer at 150–250 µL per 20 mg tissue. If lysis is insufficient, increase the volume appropriately; if a higher-concentration protein sample is required, the volume can be reduced.

(4) Homogenize on ice 30–50 strokes using a glass homogenizer, or sonicate to disrupt the cells (30 s each time, 3–4 times, with 1-min intervals on ice for cooling). After disruption, check under a microscope: the cell lysis rate should be no less than 90%, and tissue samples should be fully lysed with no obvious fragments.

(5) Transfer

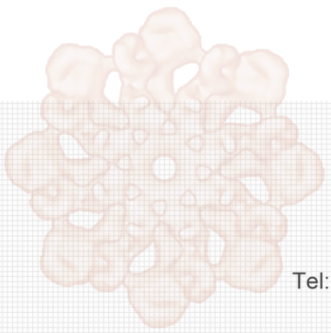
the homogenate to a centrifuge tube and incubate on ice for 5–10 min, vortexing vigorously 3–4 times for 30 s each to ensure complete lysis.

(6) After complete lysis, centrifuge at 14,000 × g at low temperature for 5 min. Collect the supernatant for subsequent WB, IP, and PAGE assays.

***Note:** For every 20 mg of frozen mouse liver tissue, lysis with 200 µL of this buffer typically yields a supernatant protein concentration of about 15–25 mg/mL. Actual concentrations vary with tissue condition and type.

Note

1. All steps of sample lysis must be performed on ice or at 4°C.
2. To obtain optimal performance, avoid repeated freeze–thaw cycles. It is recommended to aliquot the buffer appropriately before use.
3. Although this product already contains general protease and phosphatase inhibitors, for better inhibition you may prepare your own, or purchase APExBIO Protease, Phosphatase, and Deacetylase Inhibitor Cocktails.
4. SDS in the lysis buffer tends to precipitate at 4°C. Before use, warm at 37°C in a water bath to fully dissolve it, then bring back to room temperature for use.
5. RIPA lysis buffer contains ionic detergents; it may not be suitable if the kinase of interest is easily denatured.
6. Do not add phosphatase inhibitors when preparing lysates intended for phosphatase activity assays.
7. Because this product contains a relatively high concentration of detergents, the Bradford assay is not recommended for protein quantitation. Instead, use the BCA method (standard BCA, K4101, range 50–2000 µg/mL; or Micro BCA, K4102, range 0.5–20 µg/mL) or a detergent-compatible Bradford assay (K4104, range 0.1–1.5 mg/mL).
8. A small transparent gel-like mass often appears in RIPA lysates; this is normal. It is a complex containing genomic DNA and other components. If you are not analyzing proteins that bind very tightly to genomic DNA, you can simply centrifuge and take the supernatant for subsequent experiments. If you need to detect proteins that are tightly associated with genomic DNA, sonicate to disperse this gel-like material and then centrifuge and collect the supernatant. For many common transcription factors such as NF-κB or p53, sonication is usually not necessary to detect them.
9. If the tissue sample is very small, it can be cut into small pieces and lysed directly in lysis buffer, with vigorous vortexing to ensure complete lysis. Then centrifuge and collect the supernatant for downstream experiments as usual.
10. This product is for research use only.



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