Production: Cell/Tissue Lysis Buffer
Catalog No: RC0005

Product Information

The extraction of protein from cells or tissues is one of the key factors that affect the results of Western blotting analysis (WB).In practice,the extraction of protein from cell membranes, cytoplasm, organelles, and nuclei usually uses detergent-based buffers such as radioimmunoprecipitation assay (RIPA) buffer, physical disruption such as sonication, or a combination of both, in particular, RIPA buffer containing 0.1% SDS or its substitutes (such as NP-40 buffers without SDS) have been widely used as a standard method for the lysis of mammalian cells and tissues.In fact, RIPA can effectively dissolve and extract most of the proteins of medium and small molecules with a molecular weight of less than 90 kDa, but it is not effective in extracting large proteins with a molecular weight of greater than 90 kDa. In order to extract large proteins more effectively, many laboratories use a combination of RIPA buffer and sonication to physically break down DNA to reduce the viscosity of the lysates. However, sonication has the potential to break down large proteins. In addition, in order to inhibit endogenous enzyme activities, inhibitors need to be added to the RIPA buffer.For example, to reduce protein degradation, protease inhibitors such as aprotinin, leupeptin, pepstatin, and PMSF need to be added to RIPA buffer immediately before use. Similarly, sodium fluoride and sodium orthovanadate must be added to inhibit phosphatase activities.
Our Cell/Tissue Lysis buffer solve these issues. It can quickly and completely extract proteins from cells and tissues,avoiding adding protease, phosphatase, and other enzyme inhibitors; it can also preserve the post-translational modifications (PTMs) of the cellular proteins. Overall, this product is suitable for extracting proteins of all sizes from mammalian cells and tissues.

Components

Components	Size (20mL)	Size (50mL)	Size (100mL)
Reagent A	40 µL	100 µL	200 µL
Reagent B	20 mL	50 mL	100 mL

Key Features

1. No proteases/other enzyme inhibitors or sonication required.
2. Simply mix A and B;Extraction takes only 15 min
3. Extract large proteins nearly completely; no sonication to avoid protein fragmentation.
4. No loss of protein PTMs such as phosphorylation, glycosylation, ubiquitination, methylation, and acetylation
5. Suitable for mammalian cells and tissues.

Storage

Store Reagent A at -20°C. Store Reagent B at room temperature or 4°C
Note: Precipitation may occur when Reagent B is stored at 4 °C over a long time, but it does not affect product quality. The precipitation will redissolve at room temperature.

Application

Denaturing protein extraction; Western blotting

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Operation Steps

For Adherent Cell

1. Adding 2 μL of Reagent A into 1 mL of Reagent B immediately before use(Ratio of Reagent A and B: 500:1). Mix thoroughly by vortexing and place on ice
   Note: Calculate the volume of the lysis buffer you need in advance according to Step 3; discard the unused buffer after use.
2. Discard the cell culture medium and wash the cells twice with ice-cold PBS.
3. Place the culture dish/plate on ice or ice water and add 1.5 mL of the premixed lysis buffer per $5x10^6$ cells (e.g. add 300 μL of lysis buffer to a 35 mm dish containing $1x10^6$ cells). Keep the dish/plate on ice for an additional 5 min and swirl occasionally to allow the lysis buffer to fully cover the cells.
4. After 5 min of lysis, scrape the cells off the dish/plate by a clean plastic scraper and collect the lysate into a centrifuge tube.
5. Vortex the lysates thoroughly (3 x 10 sec) and place the lysates on ice or ice water for another 10 min to complete the lysis.
6. Heat the lysates on a 95°C heat block for 5 min.
7. Cool the lysates on ice or ice water for 3 min.
8. Centrifuge the lysates at 13,000g for 5 min at 4°C.
9. Measure the protein concentration using a spectrophotometer or SDS compatible protein assay.
10. If for further use, store the lysates at -20°C.
    Note: For reducing SDS-PAGE, a final concentration of 2–5% β-mercaptoethanol or 50 mM DTT, plus 0.1% bromophenol blue, must be added to the lysates. Samples should be heated at 95°C for 5 min before loading

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For Suspension Cell

1. Prepare Cell/Tissue Lysis buffer immediately before use according to Step 1 in the adherent cell protocol.
2. Centrifuged the suspended cells at 300 g for 5 min, discard the supernatant, and resuspend the cells with 10 mL of ice-cold PBS. Centrifuge again, discard the PBS, and resuspend the cells into the residual PBS with a pipette.
3. Add 1.5 mL of premixed Cell/Tissue Lysis buffer to per $5x10^6$ cells, mix well through a pipette, and then place in ice or ice water for 5 min.
4. Follow steps 5-10 in the adherent cell protocol.

For Tissue Protein

1. Prepare Cell/Tissue Lysis buffer immediately before use according to Step 1 in the adherent cell protocol.
2. In liquid nitrogen, grind tissue into fine particles using a mortar and pestle.
3. Add the frozen tissue powder into the premixed Cell/Tissue Lysis buffer at the ratio of 1g of tissue to 3 mL of lysis buffer.
4. Using a homogenizer to homogenize the tissue.
   Note: keep the tubes on the ice when homogenizing to avoid homogenization heats the sample
5. Incubate homogenized samples on ice for at least 15 min for complete lysis.
   Note: If there are multiple samples, keep all homogenized samples on ice until the last sample is done.
6. 15 min after homogenization of the last sample, centrifuge at 13,000 g at 4°C for 10 min. Transfer the supernatant with extracted proteins to a clean centrifuge tube.
7. Follow Steps 6-10 in the adherent cell protocol.