LIFESCT
Life Scientists' Service Center
Email: support@lifesct.com
Tel: +1-240-715-2985 Fax: +1-240-252-7376
9610 Medical Center Drive, Rockville, MD 20850, USA

IntactProtein™ Cell-Tissue Lysis Kit

Cat. #: C0002 Size: 20 ml

Introduction

One of the key factors influencing Western blot results is the extraction of proteins from cells. In practice, detergent-based buffers such as radioimmunoprecipitation assay (RIPA) buffer, along with physical disruption such as sonication, or the combination of both, have become the norm for protein extraction from cell membranes, cytoplasm, organelles, and nuclei.

Although RIPA buffer (with 0.1% SDS) or its alternative such as NP-40 buffer (without SDS), has been widely used to lyse cultured mammalian cells, RIPA buffer is not as efficient at extracting large-sized proteins compared to medium- and small-sized peptides. To increase the harvest of large-sized proteins, most labs combine RIPA buffer with sonication which can physically break down DNA and thus reduce the viscosity of the lysates. Yet, sonication can break down the large-sized proteins. Additionally, to inhibit protease and phosphatase activities, inhibitors must be added to the RIPA buffer.

IntactProtein™ Cell-Tissue Lysis Kit is formulated to solve these issues. It saves you time by 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.

Package Information

Components	C0002
Reagemt A	40 µl
Reagemt B	20 ml

Storage

Reagemt A at -20°C; Reagent B at room temperature

Protocol

Protein Extraction from Adherent Cells

1. Prepare the IntactProtein™ lysis buffer by adding 2 µl of Reagent A into 1 ml of Reagent B immediately before use. Mix thoroughly by vortexing and place on ice.

Note: Calculate the volume of the lysis buffer you need as per 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 ml of the premixed lysis buffer per 5×10^6 cells (e.g. add 300 µl of lysis buffer to a 35 mm dish containing 1×10^6 cells). Keep the dish/plate on ice for an additional 5 min and swirl occasionally to allow the lysis buffer to completely cover the cells.

4. After 5 min of lysis, scrape the cells off the dish/plate using a clean plastic scraper and collect the lysate into a centrifuge tube.

5. Vortex the lysates thoroughly (3× 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 13,000 g for 5 min at 4°C.

9. Measure the protein concentration using a NanoDrop spectrophotometer or SDS-compatible protein assay.

10. Store the lysates at -20°C for future use or use immediately for further analysis.

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.

Protein Extraction from Suspension Cells

1. Prepare the IntactProtein™ lysis buffer immediately before use as described in Step 1 of the Protocol for Adherent Cells.

2. Centrifuge suspension cells at 300g for 5 min and resuspend in 10 ml of ice-cold PBS. Centrifuge again, discard the PBS, and resuspend the cells into the residual PBS by pipetting.

3. Add 1 ml of the premixed IntactProtein™ lysis buffer per 5×10^6 cells directly into the resuspended cells. Mix well by pipetting and place on ice or ice water for 5 min.

4. Follow Steps 5-10 in the Protocol for Adherent Cells.

Protein Extraction from Tissues

1. Prepare IntactProtein™ lysis buffer immediately before use as described in Step 1 of the Protocol for Adherent Cells.

2. In liquid nitrogen, grind tissue into fine particles using a mortar and pestle.

3. Add the frozen tissue powder into the premixed IntactProtein™ lysis buffer at the ratio of 1 g of tissue to 3 ml of lysis buffer.

4. Homogenize the tissue using a homogenizer as per the manufacturer's instructions.

Note: Homogenization heats the sample, so always keep the tubes on the ice.

5. Incubate homogenized samples on ice for >15 min for complete lysis.

Note: If you have 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 Protocol for Adherent Cells.