INTACTPROTEIN™ Cell/Tissue Lysis Buffer

1. KIT COMPONENT

Cat. #	Reagent A	Reagent B
AR100093S	40 µL	20 mL
AR100093	100 µL	50 mL

2. BACKGROUND

One key factor influencing the result of Western blotting is the quality of proteins extracted from cells and tissues. In practice, detergent-based buffers such as radioimmunoprecipitation assay (RIPA) buffer, physical disruption such as sonication, or a combination of both have become the standard for the extraction of proteins from cell membranes, cytoplasm, organelles, and nuclei.

Although RIPA buffer (with 0.1% SDS) or its substitute, like NP-40 buffer (without SDS), has been widely used to lyse mammalian cells and tissues, RIPA buffer is not as effective in extracting large proteins as it is in medium and small proteins. To increase the harvest of large proteins, most laboratories combine RIPA buffer with sonication, which can physically break down DNA and reduce the viscosity of the lysates. However, sonication has the potential to break down large proteins. Furthermore, inhibitors must be added to the RIPA buffer to inhibit endogenous enzyme activities. For example, to reduce protein degradation, protease inhibitors such as aprotinin, leupeptin, pepstatin, and PMSF need to be added to the RIPA buffer immediately before use. Similarly, sodium fluoride and sodium orthovanadate must be added to inhibit phosphatase activities.

Our IntactProtein Cell-Tissue Lysis Buffer 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.

3. PRODUCT FEATURES

- **All-in-one formula:** no protease/other enzyme inhibitors or sonication required
- **Ready-to-use protocol:** simply mix Reagents A & B; extraction takes as little as 15 min
- **Ultimate solution for large proteins:** near-complete extraction of large proteins; no fragmentation due to no sonication
- **Assurance and peace of mind:** no loss of protein PTMs such as phosphorylation, glycosylation, ubiquitination, methylation, and acetylation
- **All-around performance:** suitable for mammalian cells and tissues

4. STORAGE

Upon receipt, store Reagent A at -20°C. Store Reagent B at room temperature or 4°C.

Technical Notes:

1. Reagent B can be stored at room temperature for up to 3 months. For long-term storage, keep the reagent at 4 °C.
2. Precipitation may occur when Reagent B is stored at 4 °C over a prolonged period of time.
3. Precipitation does not affect product quality.
4. Precipitation will redissolve at room temperature; after the precipitation disappears, gently mix the solution.

5. APPLICATIONS

Denaturing protein extraction; Western blotting

6. EXPERIMENTAL PROTOCOL FOR 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.
   Tips: 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 $5x10^6$ 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 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 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 NanoDrop spectrophotometer or SDS- compatible protein assay.
10. Store the lysates at -20°C for future use or use immediately for further analysis.
    Tips: For reducing SDS-PAGE, a final concentration of 2–5% β-mercaptoethanol or 50 mMDTT, plus 0.1% bromophenol blue, must be added to the lysates. Samples should be heated at 95°C for 5 min before loading.

7. EXPERIMENTAL PROTOCOL FOR SUSPENSION CELLS

1. Prepare the IntactProtein lysis buffer immediately before use as described in Step 1 of the Experimental 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 $5x10^6$ 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 Experimental Protocols for Adherent Cells.

8. EXPERIMENTAL PROTOCOL FOR TISSUES

1. Prepare IntactProtein lysis buffer immediately before use as described in Step 1 of the Experimental 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.
   Tips: homogenization heats the sample, so always keep the tubes on the ice.
5. Incubate homogenized samples on ice for >15 min for complete lysis. Tips: 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 Experimental Protocol for Adherent Cells.

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