IntactProtein™ Cell-Tissue Lysis Kit

Description

IntactProtein™ cell-tissue lysis kit includes a uniquely formulated protein lysis and extraction buffer mix. This innovative and proprietary reagent mix is the first of its kind and is simple to use. It is an extraction buffer with widespread applications that eliminates the uncertainty behind choosing the correct buffer to effectively detect your protein of interest.

Key Features

- **All-in-one formula:** No protease/PTM inhibitors needed; no sonication required
- **Ready-to-use protocol:** Simply mix Reagents A&B; extraction in as little as 15 min
- **Ultimate solution for large proteins:** Near-complete extraction; no fragmentation
- **Assurance and peace of mind:** No loss of protein PTMs such as phosphorylation
- **All-around performance:** Suitable for mammalian cells and tissues

Products

Cat. #	Reagent A	Reagent B	Price
415S	40 µL	20 mL	$70
415M	100 µL	50 mL	$120
415L	200 µL	100 mL	$200

Protocols

Experimental Protocols 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. Vortex the solution to mix well.
2. Discard culture medium and wash the cells twice with ice-cold PBS.
3. With the dish/plate on ice, add 1 mL of the mixed cell lysis buffer per $5x10^6$ cells (e.g. add 300 µL of lysis buffer to a 35 mm dish). Keep the plate/dish on ice for an additional 5 min and swirl occasionally to spread the lysis buffer.
4. After 5 min of lysis, scrape the cells off the plate/dish and collect the lysate in a centrifuge tube.
5. Vortex the lysates (3 x 10 sec) and place the cells on ice for an additional 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 for 3 min.
8. Centrifuge the lysates at 13,000g for 5 min at 4C.
9. Measure the protein concentration using a NanoDrop spectrophotometer or an SDS-compatible protein assay method.
10. Store the cell lysates at -20°C or immediately use the lysates for further analysis. Note: For reducing gels, a final concentration of 2–5% β-mercaptoethanol or 50 mM DTT should be added to the lysates. The samples must be heated at 95°C for 5 min before loading.

Experimental Protocols for Suspended Cells

1. Prepare the IntactProtein™ buffer by adding 2 µL of Reagent A into 1 mL of Reagent B immediately before use. Vortex the solution to mix well.
2. Pellet the cells by centrifugation (200g for 5 min) and resuspend the cells in 10 mL of ice-cold PBS. Pellet down the cells again, discard the PBS, and resuspend the cells in the residual buffer by pipetting.
3. Add 1 mL of cell lysis buffer per $5x10^6$ cells directly to the resuspended cells. Mix by pipetting.
4. Follow steps 5-10 in the Experimental Protocols for Adherent Cells.

Experimental Protocols for Tissues

1. In liquid nitrogen, grind tissues into fine particles with a mortar and pestle.
2. Prepare the IntactProtein™ lysis buffer by adding 2 µL of Reagent A into 1 mL of Reagent B immediately before use. Vortex the solution to mix well.
3. Add the frozen tissue powder into the pre-mixed IntactProtein™ lysis reagent at the ratio of 1g of tissue to 3 mL of lysis reagent.
4. Homogenize the tissue according to the manufacturer's instructions. Tip: homogenization will heat up your sample, so always keep the tubes on ice.
5. Incubate the tissue on ice for 15 min. Tip: If you have multiple samples, keep all the samples on ice. Begin the 15 minutes after the last sample in the group is homogenized.
6. Centrifuge at 4°C for 10 min and transfer the supernatants into clean centrifuge tubes. Supernatants contain the extracted proteins.
7. Follow steps 6-10 in the Experimental Protocol for Adherent Cells.

STORAGE

Store Reagent A at –20°C; store Reagent B at room temperature

VALIDATION

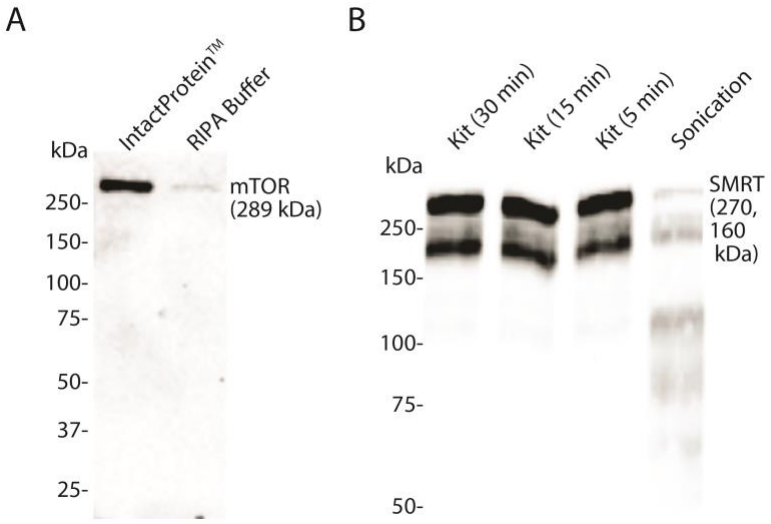

Figure 1. IntactProtein™ lysis kit demonstrates superior performance in extracting large-sized proteins compared to RIPA buffer. (**A**) HeLa cells were lysed using the IntactProtein™ lysis kit or RIPA buffer. Total lysates (50 µg) were subjected to immunoblotting using an anti-mTOR (289 kDa) antibody. (**B**) HT1080 cell lysates (50 µg) were lysed on ice, processed with the times indicated, and blotted with an anti-SMRT (160, 270 kDa) antibody. Note that sonication fragmented SMRT into smaller peptides.

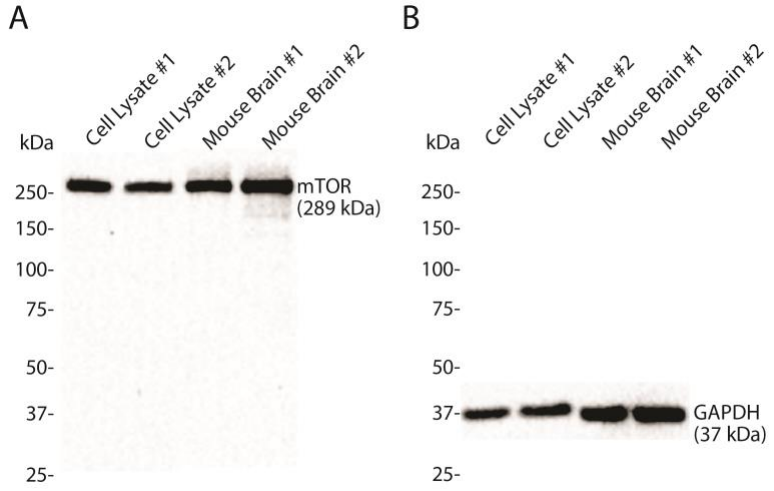

Figure 2. IntactProtein™ lysis kit performs well in extracting proteins from tissues and cells. (**A**) HeLa cells and mouse brains (in duplicate) were lysed using the IntactProtein™ lysis kit. Total lysates (50 µg) were subjected to immunoblotting using an anti-mTOR (289 kDa) antibody. (**B**) The same lysates in (**A**) were blotted with an anti-GAPDH (37 kDa) antibody. Note that the IntactProtein™ lysis kit is suitable to extract proteins of large- and small-molecular weights from both cells and tissues.

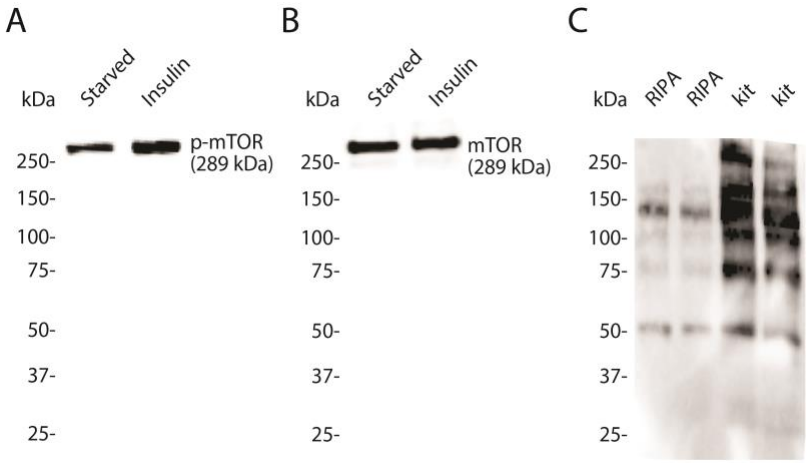

Figure 3. IntactProtein™ lysis kit preserves protein post-translational modifications (PTMs). (**A-B**) HeLa cells were serum starved for 16 h and stimulated with insulin (150 nM) for 5 min before protein extraction using the IntactProtein™ lysis kit. Total lysates (50 µg) were subjected to immunoblotting using anti-phospho-mTOR (Ser2448) (**A**) and anti-mTOR (**B**) antibodies, respectively. (**C**) HeLa cells were lysed using RIPA buffer or the IntactProtein™ lysis kit. Cell lysates were immunoblotted using anti-O-Linked β-N-acetylglucosamine (O-GlcNAc) (glycosylation) antibody.