ClearBand RIPA Lysis Buffer, Complete System

50 ml RIPA Lysis Buffer, 0.5 ml 100x Protease Inhibitor Cocktail, 0.5 ml 100x PMSF

Cat No: RIPA50CS

Shipping **:** Ship with blue ice.
Storage **:** Store RIPA Lysis Buffer at 4°C. Store 100x Protease Inhibitor Cocktail and 100x PMSF at –20°C. Store RIPA Lysis Buffer supplemented with Protease Inhibitor Cocktail and 100x PMSF at -20°C.

General Information

ClearBand RIPA Lysis Buffer, Complete System is a convenient and effective solution designed for the extraction of total protein from cultured cells and tissue samples. The system combines a ready-to-use RIPA Lysis Buffer with 100X Protease Inhibitor Cocktail and 100X PMSF, providing a practical format for efficient protein extraction while helping protect proteins from proteolytic degradation during the lysis process.

The inclusion of protease inhibitors in this complete system helps preserve protein integrity during sample preparation, especially when working with sensitive targets. *ClearBand* RIPA Lysis Buffer, Complete System is suitable for routine laboratory use in protein extraction workflows prior to applications such as Western blotting, protein quantification assays, immunodetection, and other biochemical analyses.

Principle

ClearBand RIPA Lysis Buffer, Complete System is a strong lysis buffer commonly used to disrupt cell membranes and release intracellular proteins. In combination with protease inhibitors, it enables efficient extraction of proteins while minimizing degradation during sample processing. The complete system is intended to simplify lysate preparation by providing the essential reagents required for immediate use.

Preparation Before Use

Before starting, prepare the working lysis buffer by adding inhibitors to the required amount of *ClearBand* RIPA Lysis Buffer.

Recommended Working Solution

For each **1 mL of *ClearBand* RIPA Lysis Buffer**, add:

- **10 µL of 100X Protease Inhibitor Cocktail**
- **10 µL of 100X PMSF**

Mix gently and keep the buffer **cold** during use.

Note: Prepare only the amount needed for immediate use when possible.

For further information,
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Protocol

A. Protein Extraction from Cultured Cells

Adherent Cells

1. Remove culture medium completely. Gently wash the cells with ice-cold PBS to remove residual medium.
2. Add appropriate volume of ice-cold working ***ClearBand*** RIPA Lysis Buffer directly to the plate.
    - **Suggested volume:** 100–300 µL per well of a 6-well plate
    - Adjust volume depending on cell density and culture vessel size.
3. Scrape the cells thoroughly and transfer the lysate to a pre-cooled microcentrifuge tube.
4. To increase yield, snap freeze and thaw samples 2-3 times until all the cells burst.
5. Incubate on ice for 15 minutes, vortexing briefly every 5 minutes.
6. Centrifuge at 12,000–14,000 × g for 10 minutes at 4°C.
7. Carefully transfer the clear supernatant to a new pre-cooled tube, quantify, add ***ClearBand*** Laemmli Sample Buffer, and boil for 5 minutes.

Suspension Cells

1. Collect cells by centrifugation at 300–500 × g for 5 minutes. Discard the supernatant and wash the pellet once with ice-cold PBS.
2. Centrifuge again and remove residual PBS.
3. Resuspend the cell pellet in ice-cold working ***ClearBand*** RIPA Lysis Buffer.
    - **Suggested starting volume:** 100–500 µL per 1×10^6 to 5×10^6 cells
4. Pipette up and down to resuspend. To increase yield, snap freeze and thaw samples 2-3 times until all the cells burst.
5. Incubate on ice for 15 minutes, vortexing briefly every 5 minutes.
6. Centrifuge at 12,000–14,000 × g for 10 minutes at 4°C.
7. Carefully transfer the clear supernatant to a new pre-cooled tube, quantify, add ***ClearBand*** Laemmli Sample Buffer, and boil for 5 minutes.

B. Protein Extraction from Tissue Samples

1. Keep tissue samples cold at all times. Weigh the tissue sample.
    - **Suggested starting ratio:** 500–1000 µL per 100 mg tissue

    a. Grind the sample in a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw. Immediately, add working ***ClearBand*** RIPA Lysis Buffer to grinded tissue. Transfer the grinded and homogenized tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Mix well by vortexing for 10-15 seconds.

    b. Cut the tissue sample and determine the amount of tissue by weighing. Transfer the tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Add working ***ClearBand*** RIPA Lysis Buffer. Immediately and vigorously homogenize using a conventional rotor-stator homogenizer with a stainless-steel probe at 15,000 rpm for 30 seconds.
2. To increase yield, snap freeze and thaw samples 2-3 times until all the cells burst. Keep samples in ice for 10-15 minutes to dissolve structures and complexes, mixing occasionally.
3. Centrifuge at 12,000–14,000 × g for 10 minutes at 4°C.
4. Transfer the clarified supernatant to a new pre-cooled tube without disturbing debris, quantify, add ***ClearBand*** Laemmli Sample Buffer, and boil for 5 minutes.

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Important Notes

- Keep all reagents and samples **cold** during the extraction procedure.
- Add **Protease Inhibitor Cocktail** and **PMSF** before use for best protection against protein degradation.
- PMSF is unstable in aqueous solutions; therefore, it is recommended to add it to the working buffer shortly before use.
- Lysate viscosity may increase if genomic DNA is released; in such cases, brief sonication or repeated pipetting may help reduce viscosity.
- For some proteins, lysis efficiency may vary depending on sample type and target localization; optimization may be required.
- Avoid repeated freeze-thaw cycles of prepared lysates.

Storage of Lysates

- For short-term use: store lysates on ice during handling
- For longer storage: aliquot and store at -20°C or preferably -80°C

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