BIO BASIC
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Product information

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RIPA Buffer II

Catalog #: PL006
Size: 10 preps
Storage: Mixed components storage*

*: Product will be shipped with ice pack. Check storage conditions.

Product Description:

RIPA Buffer II is one of the most reliable buffers with middle-strong strength to lyse cultured mammalian cells from both plated and pelleted cells to suspension cultures. This buffer enables protein extraction from cytoplasmic, membrane and nuclear proteins and is compatible with many applications, including reporter assays, protein assays, immunoassays and protein purification. The buffer is also supplied with additional protease inhibitor and phosphatase inhibitor buffer to maintain protein integrity and high biological activity. Depending on the downstream applications, additional agents such as reducing agents and/or EDTA, EGTA may be added into the RIPA Buffer II, 10 ml is sufficient for$10x10^7$ cells or 10x100mg tissue sample.

Storage and Transportation:

Upon receipt, store RIPA Buffer II at room temperature and keep Protease inhibitor buffer and Phosphatase inhibitor at -20°C.

Composition:

RIPA Buffer II	10 ml
Protease Inhibitor Buffer	10 µl
Phosphatase Inhibitor Buffer	50 µl

Procedures:

1. Procedure for Lysing Cell Monolayer (Adherent) Cultures:

1. Carefully remove culture medium from cells. Wash the cells once with ice cold PBS for three times.
2. Add ice cold RIPA Buffer II (before use, add 1 µl Protease inhibitor buffer and 5 µl Phosphatase inhibitor buffer into 1 ml RIPA Buffer II) to the cells according to the table below and incubate on ice for 10 minutes with periodic mixing and uniform spreading, then Pipette the mixture up and down to help lysis.

Plate Size	Volume of RIPA Buffer II
100 × 100 mm	500-1,000 µl
100 × 60 mm	250-500 µl
6-well plate	200-400 µl per well
24-well plate	100-200 µl per well

NOTE: For T-25 flask and T-75 flask, use 1 ml of ice cold RIPA Buffer II per 75 cm^2 flask containing $5x10^6$ HeLa or A431 cells. Keep on ice for 5 minutes, swirling the plate occasionally for uniform spreading. Scrape the lysate to one side using a cell scraper, collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at ~14,000 g for 15 minutes to pellet the cell debris. To increase yields, sonicate the pellet for 30 seconds with 50% pulse.

3. Collect and transfer the lysate to a microcentrifuge tube and centrifuge at ~13,000 × g for 10 minutes to pellet the cell debris at 4°C.
4. Transfer supernatant to a new tube for protein concentration determination and further analysis.

2. Procedure for Lysing Cell Suspension Cultures:

1. Centrifuge the cell suspension at 1,000 × g for 5 minutes to pellet the cells. Discard the supernatant.
2. Wash the cells once with ice cold PBS. Centrifuge at 1,000 × g for 5 minutes to pellet cells.
3. Add ice cold RIPA Buffer II to the cell pellet. Use 500 µl of RIPA Buffer II (before use, add 1 µl Protease inhibitor buffer and 5µl Phosphatase inhibitor buffer into 1 ml RIPA Buffer II) for per 50 mg of wet cell pellet. Pipette the mixture up and down to suspend the pellet.

   NOTE: If using large amount of cells, first add 10% of the final volume of RIPA Buffer II to the pellet and pipette the mixture up and down to mix. Add the remaining volume of RIPA Buffer II to the cell suspension.

   NOTE: To increase yields, sonicate the pellet for 30 seconds with 50% pulse.
4. Incubate lysate on ice for 15 minutes with periodic mixing. Remove cell debris by centrifugation at ~13,000 × g for 10 minutes at 4°C.
5. Transfer supernatant to a new tube for protein concentration determination and further analysis.

3. Procedure for Lysing Animal Tissue:

1. For tissue sample, one extraction requires approximately 100 mg of sample. Remove fat and nerve tissue at best, cut it into small pieces, and then wash them with pre-cold PBS for three times.
2. Add 1 ml ice cold RIPA Buffer II (before use, add 1 µl Protease inhibitor buffer and 5 µl Phosphatase inhibitor buffer into 1 ml RIPA Buffer II), vortex, homogenize them with glass homogenizer for 30-50 strokes or sonicate for 30 seconds, interval 1 minute, repeat operation for three times. Check the efficiency of cell fracture and ensure more than 90 percent cells have been broken.
3. Transfer the above homogenization buffer into a new 1.5 ml pre-cold centrifuge tube, place them on icebag for 10 minutes, occasionally vortex for 3-4 times, then centrifuge at 18000 × g for 5 minutes at 4°C, discard precipitates and remain supernatant for IP assays, protein assays, reporter assays and other immunoassay procedures.

Notes:

1. All of reagents and instruments must be pre-cold.
2. If protein concentration is low, please increase the number of strokes performed with the homogenizer.