BIO BASIC
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Product information

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Denaturing Cell Lysis Buffer

Catalog #: PL014
Size: 10 preps
Storage: -20°C*

*: Product will be with ice pack. Check storage conditions. Products have one year expiration from time of purchase.

Product Description:

The denaturing Cell Lysis Buffer is suitable for cell lysis of the following proteins: (1) The insoluble antigen, such as cytoskeleton and membrane protein, hydrophobic protein. (2) The proteins, epitopes of which are hidden in secondary even higher structure and some proteins were subjected to aggregate and precipitate. (3) The proteins translated in vitro, treated protein can be used for downstream experiments such as SDS-PAGE, 1D & 2D electrophoresis, 10 ml of the Buffer is sufficient for $10x10^7$ cells or 10x100 mg tissue sample.

Storage and Transportation:

After received, store Lysis Buffer and Protease Inhibitor Buffer at -20°C.

Composition:

Lysis Buffer	10 ml
Protease Inhibitor Buffer	100 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 Lysis Buffer (before use, add 10 µl Protease Inhibitor Buffer into 1 ml Lysis Buffer) to the cells according to the table below and incubate at room temperature for 10 minutes with periodic mixing for uniform spreading, then pipette the mixture up and down to help lysis.

Plate Size	Volume of Lysis Buffer
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 Lysis Buffer per 75 cm^2 flask containing 5x 10^6 HeLa or A431 cells. Keep at room temperature for 5 minutes, swirling the plate occasionally for uniform spreading. Gather the lysate to one side using a cell scraper, collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at ~14,000 x 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 x g for 10 minutes to pellet the cell debris at room temperature.

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 x g for 5 minutes to pellet the cells. Discard the supernatant.

2. Wash the cells once with ice cold PBS. Centrifuge at 1,000 x g for 5 minutes to pellet cells.

3. Add Lysis Buffer to the cell pellet. Use 500 µl of Lysis Buffer (before use, add 10 µl Protease Inhibitor Buffer into 1 ml Lysis Buffer) for per 50 mg of wet cell pellet, Pipette the mixture up and down to suspend the pellet.

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

NOTE: To increase yields, sonicate the pellet for 30 seconds with 50% pulse.

4. Incubate lysate at room temperature for 15 minutes with periodic mixing. Remove cell debris by centrifugation at ~13,000 x g for 10 minutes at RT.

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 needs 100 mg, remove fat and nerve tissue at beat, cut it into small pieces, and then wash them with pre-cold PBS for three times.

2. Add 1 ml Lysis Buffer (before use, add 10 µl Protease Inhibitor Buffer into 1 ml Lysis Buffer), 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, must more than 90 percent cells have been broken.

3. Transfer the above homogenization buffer into a new 105 ml pre-cold centrifuge tube, place them at room temperature for 10 minutes, occasionally vortex for 3-4 times, then centrifuge at 18,000 x g for 5 minutes at RT, discard precipitates and remain supernatant for SDS-PAGE, 1D & 2D electrophoresis.

Notes:

1. If Lysis Buffer at -20°C have precipitates at the bottom of bottle, please heat to 37°C to completely dissolve them before use.

2. If low protein concentration, maybe cell lysis was not efficient, please increase the number of strokes performed with the homogenizer.

3. For protein quantization, can use Non-Interfering Protein Concentration Determination Kit (SK3071) or modified Lowry Protein Assay Kit (SK4041).