BIO BASIC
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Product information

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IP/Western Lysis Buffer

Catalog #: PL035
Size: 10 preps
Storage: Mixed components storage*

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

Product Description:

The IP/Western Lysis Buffer is optimized for cell lysate yield, purity and compatibility with immunoprecipitation. The Buffer is a mammalian whole cell lysis reagent based on a modified RIPA buffer formulation without SDS. This Ready-made formula is effective for extracting cytoplasmic, membrane and nuclear proteins. The kit does not liberate genomic DNA or disrupt protein complexes like ordinary RIPA buffer and it helps maintain protein complexes for co-immunoprecipitation. The Buffer is specially formulated and designed for pull down and IP assays, protein assays, reporter assays and other immunoassay procedures, in addition, the buffer contains additional protease inhibitor and phosphatase inhibitor to maintain protein's biology activity. 10 ml is sufficient for $10x10^7$ cells or 10x100 mg tissue sample.

Storage and Transportation:

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

Composition:

Lysis Buffer	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 lysis buffer (before use, add 1 µl Protease inhibitor buffer and 5 µl Phosphatase inhibitor buffer into 1 ml lysis buffer) to the cells according to the table below and incubate on ice for 5 minutes with periodic mixing.

Plate Size	Volume of IP/Western 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

3. 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 IP/Western Lysis Buffer to the cell pellet. Use 500 µl of lysis buffer (before use, add 1 µl Protease inhibitor buffer and 5 µl Phosphatase inhibitor buffer into 1 ml lysis buffer) per 50 mg of wet cell 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.

4. Incubate lysate on ice for 5 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 needs 100 mg, 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 IP/Western Lysis Buffer (before use, add 1 µl Protease inhibitor buffer and 5 µl Phosphatase inhibitor buffer into 1 ml lysis buffer), vortex, homogenize with a glass homogenizer for 30-50 strokes or sonicate them for 30 seconds, 1 minute interval. Repeat operation three times. Check the efficiency of cell lysis 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 ten minutes. Occasionally vortex for 3-4 times and then centrifuge at 18000 × g for 5 minutes at 4°C. Discard precipitates and keep supernatant for IP assays, protein assays, reporter assays and other immunoassays.

Notes:

1. All of reagents and instruments must be pre-cold to maintain protein integrity and activity.
2. If low protein concentration is observed, try to increase the number of strokes performed with the homogenizer.