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Product information

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Non-denaturing Cell Lysis Buffer

Catalog #: PL013
Size: 10 preps
Storage: -20°C

Product Description:

The Non-denaturing Cell Lysis Buffer contains no-ion detergent, salt, EDTA. The Lysis buffer is able to lyse cells from adherent cultures, suspension cultures as well as animal tissues. The Lysis buffer enables protein extraction from cytoplasmic, membrane and nuclear proteins. Protein extracted can be readily used in many applications, including reporter assays, protein assays, immunoassays and protein purification. The Lysis Buffer is supplied with protease and phosphatase inhibitors that maintain protein integrity and high biological activity. Depending on the required downstream applications, additional agents such as reducing agents, other protease inhibitors may be added into the The Lysis Buffer, 10ml is sufficient for10x10^7 cells or 10x100mg tissue sample.

Storage and Transportation:

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

Composition:

Components	
Lysis Buffer	10ml
Protease Inhibitor Buffer	100ul
Phosphatase Inhibitor Buffer	50ul

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 10ul Protease inhibitor buffer and 5ul Phosphatase inhibitor buffer into 1ml Lysis Buffer) to the cells according to the table below and incubate on ice for 10 minutes with periodic mixing for uniform spreading. Pipette the mixture up and down a few times.

Plate Size/Surface Area	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 on ice 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 g for 15 minutes to pellet the cell to increase yields, sonicate the pellet for 30 seconds with 50% pulse

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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 Monolayer (Adherent) 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 Lysis Buffer to the cell pellet. Use 500 µl of Lysis Buffer (before use, add 10ul Protease inhibitor buffer and 5ul Phosphatase inhibitor buffer into 1ml Lysis Buffer) for per 50 mg of wet cell pellet. Pipette the mixture up and down.
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 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 need 100mg, 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 1ml ice cold Lysis Buffer (before use ,add 10ul Protease inhibitor buffer and 5ul Phosphatase inhibitor buffer into 1ml Lysis Buffer), vortex, homogenize them with glass homogenizer for 30-50 strokes or sonicated them for 30 seconds, interval 1 minutes, repeat operation for three times. Check the efficiency of cell fracture. More than 90 percent cells should have been lysed.

3. Transfer the above homogenization buffer into a new 1.5ml pre-cold centrifuge tube, place them on ice for ten minutes, occasionally vortex for 3-4 times, 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 immunoassay procedures .

Notes

1. All of reagents and instruments must be pre-cold, thus the extracted protein can remain activity and intact.

2. If low protein concentration, cell lysis may not be efficient. Please increase the number of strokes performed with the homogenizer.

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