



Product Information

RIPA Buffer III

Product information for PL007:

Introductions:

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

Compositions:

Components	PL007
RIPA Buffer III	10ml
Protease Inhibitor Buffer	10ul
Phosphatase Inhibitor Buffer	50ul

Storage and Transportation:

Transportation at room temperature, after received, store RIPA Buffer III at room temperature and keep Protease inhibitor buffer and Phosphatase inhibitor buffer at -20°C.

Procedures:

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

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

Plate Size/Surface Area	Volume of RIPA Buffer III
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 III per 75 cm² flask containing 5×10^6 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 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 III to the cell pellet. Use 500 µl of RIPA Buffer III (before use ,add 1ul Protease inhibitor buffer and 5ul Phosphatase inhibitor buffer into 1ml RIPA Buffer III) 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 RIPA Buffer III to the pellet and pipette the mixture up and down to mix. Add the remaining volume of RIPA Buffer III 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 RIPA Buffer III (before use ,add 1ul Protease inhibitor buffer and 5ul Phosphatase inhibitor buffer into 1ml RIPA Buffer III), vortex, homogenize them with glass homogenizer for 30-50 strokes or sonicated them for 30 seconds, interval 1minutes,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 1.5ml pre-cold centrifuge tube, place them on icebag for ten 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 .

Notices:

1. All of reagents and instruments must be pre-cold, thus the extracted protein can remain activity and intact.
2. If low protein concentration, maybe cell lysis was not efficient, please increase the number of strokes performed with the homogenizer.