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Product No. 16488

RIPA Buffer

Features

- Ready-to-use solution.
- One of the most reliably-used buffers to lyse cultured mammalian cells.
- Preservatives used in RIPA Buffer does not affect Antigen-Antibody interaction and protein extraction.

Composition

50 mmol/L Tris-HCl Buffer (pH 7.6), 150 mmol/L NaCl, 1% Nonidet® P-40 Substitute, 0.5% Sodium Deoxycholate, 0.1% SDS

Protocol

A) For suspension cells

1. Remove medium from cultured cells, and wash cells twice with cold D-PBS.
2. Remove D-PBS and add RIPA Buffer to the cell pellet, and vortex.
   [Add RIPA Buffer at 0.5 - 5.0 X10^7 cells/1mL RIPA Buffer.]
3. Fragment the DNA by passing the lysed suspension through a needle (21 gage) attached to a syringe.
   (This step can be skipped, but the yield of proteins may be increased by DNA fragmentation.)
4. Incubate the samples for 15 minutes on ice. (To increase the yield, extend the incubation time.)
5. Transfer the lysate to a new tube, and centrifuge at 10,000 xg for 10 minutes at 4 °C.
6. Transfer the supernatant containing the total protein extracts to a new tube for further analysis.

B) For adherent cells

1. Remove medium from cultured cells, and wash cells twice with cold D-PBS.
2. Add RIPA Buffer to the culture dish, and stir slowly for 5 minutes.
   [Add RIPA Buffer at 0.5 - 5.0 X10^7 cells/1mL RIPA Buffer.]
3. Scrape the cells completely with a cell scraper.
4. Transfer the lysate and pellet to a new tube.
5. Wash the culture dish with 400 µL RIPA Buffer, and pool the solution in the collection tube.
6. Fragment the DNA by passing the lysed suspension through a needle (21 gage) attached to a syringe.
   (This step can be skipped, but the yield of proteins may be increased by DNA fragmentation.)
7. Incubate the samples for 15 minutes on ice. (To increase the yield, extend the incubation time.)
8. Transfer the lysate to a new tube, and centrifuge at 10,000 xg for 10 minutes at 4 °C.
9. Transfer the supernatant containing the total protein extracts to a new tube for further analysis.

C) For tissue

1. Chop tissue into pieces using a scalpel.
2. Add 3 mL RIPA Buffer to 1 g tissue on ice.
3. Homogenize the tissue on ice.
4. Incubate the samples for 0.5 - 1 hour on ice.
5. Transfer the lysate to a new tube, and centrifuge at 10,000 xg for 10 minutes at 4 °C.
6. Transfer the supernatant containing the total protein extracts to new tube for further analysis.

Attention

- If highly viscous substances appear during protein extraction, increase the amount of RIPA Buffer or pass the lysed suspension 5 to 10 times through a needle (21 gage) attached to a syringe.
- Add protease inhibitor cocktail or phosphatase inhibitor cocktail to RIPA Buffer as necessary.

Storage

Refrigerator (2 – 8 °C)

Expiration

Expiration date is stated on the product label.

Packing

100 ml (Product No.16488-34)

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