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Product No. 08714

RIPA Buffer (10X)

Features

- One of the most reliable buffers used to lyse cultured mammalian cells, while preventing proteolysis and interference with immunoreaction and biological activity.
- Contains Protease Inhibitor Cocktail. Not necessary to add protease inhibitors (e.g. PMSF).
- SDS solution comes with RIPA Buffer but not pre-mixed. Suitable for immunoprecipitation, where SDS may adversely affect the Antigen-Antibody reaction.
- Preservative used in RIPA Buffer does not affect Antigen-Antibody reaction and protein extraction.

Components

Reagents	Volume	Quantity	Bottle
RIPA Buffer with Protease Inhibitor Cocktail, without SDS (10X)	2 mL	5	Umber tube
SDS Solution (1% SDS)	2 mL	5	Clear tube

Required reagents

Water deionized & sterilized for Molecular Biology (Product No.06442) or Ultrapure water (protease and protein free)

Composition

1X Solution

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

Preparation

1. Thaw RIPA Buffer (10X) and SDS Solution completely at room temperature and vortex them.
2. Mix 800 µL of water, 100 µL of RIPA Buffer (10X) and 100 µL of SDS Solution in a microtube.

- When a different volume is desired, mix water : RIPA Buffer (10X) : SDS Solution = 8:1:1.
- For the preparation of RIPA buffer without SDS, mix water : RIPA buffer (10X) = 9:1.
- Store 1X RIPA buffer at -20 °C. It is recommended to use additional protease inhibitors [e.g. Protease Inhibitor Cocktail (EDTA free) (Product No.03969)] when 1X RIPA buffer is stored over a month.

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 1X RIPA buffer to the cell pellet, and vortex.
   (Add 1X 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 procedure 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 it at 10,000xg for 10 minutes at 4 °C.
6. Transfer the supernatant containing the total protein extracts to a new tube for further analysis.

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B) For adherent cells

1. Remove medium from cultured cells, and wash cells twice with cold D-PBS.
2. Add 1X RIPA buffer to the culture dish, and stir slowly for 5 minutes.
   (Add 1X 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 with pellet to a new tube.
5. Wash the culture dish with 400 µL 1X 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 it at 10,000xg for 10 minutes at 4 °C.
9. Transfer the supernatant containing 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 1X RIPA buffer to 1 g tissue on ice.
3. Homogenize the tissue cell on ice.
4. Incubate the samples for 0.5 - 1 hour on ice.
5. Transfer the lysate to a new tube, and centrifuge it at 10,000xg for 10 minutes at 4 °C.
6. Transfer the supernatant containing total protein extracts to a new tube for further analysis.

Attention

- Add Phosphatase Inhibitor Cocktail (Product No.07574) or Phosphatase Inhibitor Cocktail (EDTA free) (Product No.07575) to RIPA buffer for phosphoprotein research.
- 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.

Storage

Freezer (-20 °C)

Expiration

Expiration date is stated on the product label.

Packing

1 SET (Product No.08714-04) (for 100 mL 1X RIPA buffer)

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