DNAbiotech

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10X RIPA buffer , Ready to use

Catalog no.: DB9719

5, 10, 25 and 50 ml

Related product: PMSF (DB9662)

Intended for Research Use Only

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Diba NoAvaran Azma Company

Customer and technical support

If you have any question, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

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Quality Control

In accordance with DNAbiotech Co. Management System, each part of the product tested against predetermined specifications to ensure consistent product quality.

General description

Radioimmunoprecipitation assay (RIPA) buffer is one of the lysis buffers used to lyse cells and tissue for the radioimmunoprecipitation assay (RIPA).This reagent is more denaturing than NP-40 or Triton X-100 because it contains the ionic detergents sodium deoxycholate and SDS as active constituents and is particularly useful for disruption of nuclear membranes in the preparation of nuclear extracts. This buffer gives low background but can denature kinases. Ripa buffer can disrupt protein-protein interactions and may therefore be problematic for immunoprecipitations and pull-down assays. It is compatible with many downstream applications, including SDS-PAGE, Western blot, immunoprecipitation, ELISA and BCA assays.

Note 1: In cases where it is important to preserve protein-protein interactions or to minimize denaturation, a buffer without ionic detergents (eg SDS) and ideally without non-ionic detergents (eg Triton X-100) should be used.

Note 2: Cell lysis with detergent-free buffer is achieved by mechanical shearing, often by passing cells through a syringe tip. In these cases, a simple Tris buffer will suffice, but as noted above, buffers with detergents are required to release membrane- or cytoskeleton-bound proteins.

PMSF to a final concentration of 1mM and any other protease inhibitors shoule be added immediately before use.

Product Information

Cat #: DB9662
Volume:5, 10, 25 and 50 ml
Appearance (Color): Colorless
Appearance (Form): Liquid
Turbidity: Clear
Storage condition: -20°C

Total Procedure

1. Prepare RIPA Lysis buffer by adding PMSF or your own desired protease inhibitors to the solution
2. Pour off media from tissue culture dish into waste container
3. Wash cells twice with PBS pouring excess off into waste beaker
4. Carefully soak up any extra PBS with an absorbent.
5. Add 500 ul of RIPA lysis buffer to the culture dish.
6. Use cell scraper to scrape cells from the bottom of the dish.
7. Pass cell lysate through pipette 20 times or by vortexing to form homogeneous lysate.
8. Transfer lysate to 1.5 ml microcentrifuge tube.
9. Allow samples to stand for 5 mins in ice or at 4°C (cold room).
10. (Optional) repeat step 7-9 againe.
11. Centrifuge the resulting mixture at 14,000g for 15 mins in ice or at 4°C to separate cell debris from protein.
12. Transfer supernatant to a new tube and store at -20° C.

Preparation of lysate from cell culture

1. Place the cell culture dish on ice and wash the cells with ice-cold PBS.
2. Aspirate the PBS, then add ice-cold lysis buffer (1 mL per 10^7 cells/100 mm dish/150 cm^2 flask; 0.5 mL per $5x10^6$ cells/60 mm dish/75 cm^2 flask).
3. Scrape adherent cells off the dish using a cold plastic cell scraper, then gently transfer the cell suspension into a pre-cooled microcentrifuge tube. Alternatively cells can be trypsinized and washed with PBS prior to resuspension in lysis buffer in a microcentrifuge tube.
4. Maintain constant agitation for 30 min at 4°C.
5. Centrifuge in a microcentrifuge at 4°C. You may have to vary the centrifugation force and time depending on the cell type; a guideline is

20 min at 12,000 rpm but this must be determined for your experiment (e.g. leukocytes need a very light centrifugation).

6. Gently remove the tubes from the centrifuge and place on ice, aspirate the supernatant and place in a fresh tube kept on ice, and discard the pellet.

Preparation of lysate from tissues

1. Dissect the tissue of interest with clean tools, on ice preferably, and as quickly as possible to prevent degradation by proteases.
2. Place the tissue in round-bottom microcentrifuge tubes or Eppendorf tubes and immerse in liquid nitrogen to snap freeze. Store samples at -80°C for later use or keep on ice for immediate homogenization.
3. For a ~5 mg piece of tissue, add ~300 µL of ice cold lysis buffer rapidly to the tube, homogenize with an electric homogenizer, rinse the blade twice with another 2 x 300 µL lysis buffer, then maintain constant agitation for 2 h at 4°C (eg place on an orbital shaker in the fridge). Volumes of lysis buffer must be determined in relation to the amount of tissue present. Protein extract should not be too diluted to avoid loss of protein and large volumes of samples to be loaded onto gels. The minimum recommended concentration is 0.1 mg/mL, optimal concentration is 1–5 mg/mL).
4. Centrifuge for 20 min at 12,000 rpm at 4°C in a microcentrifuge. Gently remove the tubes from the centrifuge and place on ice, aspirate the supernatant and place in a fresh tube kept on ice. Discard the pellet.

Determination of protein concentration

1. Perform a Bradford assay, a Lowry assay or a bicinchoninic acid (BCA) assay. Bovine serum albumin (BSA) is a frequently used protein standard.
2. Once you have determined the concentration of each sample, you can freeze them at -20°C or -80°C for later use or prepare for immunoprecipitation or for loading onto a gel.

Preparation of samples for loading into gels

Denatured, reduced samples

Antibodies typically recognize a small portion of the protein of interest (referred to as the epitope) and this domain may reside within the 3D conformation of the protein. To enable access of the antibody to this portion it is necessary to unfold the protein, ie denature it.

To denature, use a loading buffer with the anionic detergent sodium dodecyl sulfate (SDS), and boil the mixture at 95–100°C for 5 min. Heating at 70°C for 5–10 min is also acceptable and may be preferable when studying multi-pass membrane proteins. These tend to aggregate when boiled and the aggregates may not enter the gel efficiently.