RIPA Buffer

Part No. RIPA-30, 5X Solution, pH 7.4; 30 ml

Cell Lysis Protocol
Note: RIPA buffer (Part No. RIPA-30) is provided as a 5X solution, 30 ml. Dilute to 1X with dH_20 prior to your experiment.

A. Cell Culture:

It is recommended that cells are cultured to 80-90% confluency prior to performing cell lysis. Cells should be washed free of serum proteins using PBS to prevent appearance of non-specific serum protein bands in downstream Western blots. Remove PBS prior to addition of RIPA buffer.

B. Cell Lysis:

Dilute sufficient 5X RIPA buffer in dH_20 to make a 1X solution. Cool RIPA buffer in ice and add protease inhibitors and phosphatase inhibitors (if required) immediately prior to cell lysis. We recommend using 300µl of RIPA Buffer solution for one to three 10 cm cell culture dishes of lysed cells. Scale accordingly for other numbers or sizes of cell culture dishes according to the surface area of the dish.

1. Lyze cells and generate a supernatant fraction as follows: Apply the ice cold RIPA Buffer solution to cells for 15 minutes: Use 300µl of RIPA Buffer for one to three 10 cm plates of cells. Scale accordingly for other numbers or sizes of plates based on the surface area of the cell culture dish.

   A simple method to dislodge and lyze adherent cells is to place the washed (and PBS removed) cell culture plates on a bed of ice. Dispense ice cold RIPA Buffer with protease/phosphatase inhibitors over the cell layer, rotate the plate by hand to cover cells with a film of RIPA Buffer, then immediately dislodge the cells with a cell scraper. Now, use a transfer pipette to siphon the cells into a 1.5ml microcentrifuge tube. This cell suspension can also be transferred sequentially over multiple plates one-by-one to collect a suspension derived from several cell culture plates.

   Tap the tube with the cell suspension vigorously five times to lyze membranes. You can also vortex the cell suspension to lyze membranes if the immediately downstream application is Western Blotting. Leave the cell suspension on ice for 15 min

2. Centrifuge the cell lysate in a cooled microcentrifuge at full speed for 15 min to partition supernatant and pellet. Collect the **supernatant fraction**, which contains extracted membrane and cytosolic proteins, and dispense this supernatant into another 1.5 ml microcentrifuge tube that is placed in ice. This supernatant corresponds to the **unfractionated supernatant fraction**. The unfractionated supernatant can be aliquoted and stored at -20°C, or -80°C for longer term storage.