



RIPA Total Protein Lysate

(Cat/No.:BC037 Size:100ml)

1. Product introduction

RIPA lysis buffer is a traditional rapid lysis buffer for cells and tissues. The main lysis components contain Triton X-100, sodium deoxycholate, and SDS. The obtained protein samples can be used for conventional Western blotting.

2. Instructions for use

1. Take an appropriate amount of lysis buffer and add protease inhibitors such as PMSF a few minutes before use.

2. For cultured cell samples:

- 1) Adherent cells: remove the culture medium and wash twice with PBS or normal saline. Add lysate at a ratio of 150-250 μ L of lysate to each well of a 6-well plate. Use a pipette several times to make sufficient contact between the lysate and the cells. Shake gently for about 5-10 minutes. After incubating on ice for 1-3 hours to fully lyse, centrifuge at 10,000- 14,000g at 4°C for 10 minutes, and take the supernatant as the protein extract.
- 2) Suspended cells: collect cells by centrifugation, and flick the cells with fingers. Add lysate at a ratio of 150-250 μ L of lysate to each well of a 6-well plate. Flick or vortex for 5-10 minutes and incubate on ice for 1-3 hours to fully lyse cells. There should be no apparent cell pellet after adequate lysis. If the number of cells is large, it must be aliquoted and then processed. After fully lysed, centrifuge at 10,000-14,000g for 10 minutes at 4°C, and take the supernatant as the protein extract.

3. For tissue samples:

- 1) The surgically excised tissue pieces were quickly placed in pre-cooled physiological saline, rinsed several times, washed with tissue blood, dried with filter paper, and cut into several smaller tissue pieces after weighing the tissue. tissue homogenizer.
- 2) According to the ratio of tissue net weight (g): lysis solution (mL) = 1:10, add the



corresponding volume of lysis solution for homogenization (if the lysis is insufficient, more lysis solution can be added appropriately, if a high concentration of protein is required) sample, the amount of lysate can be appropriately reduced).

- 3) Homogenize with a glass homogenizer until there is no visible tissue block, and incubate on ice until fully lysed. After fully disintegrating, Centrifuge at 10000-14000g at 4°C for 3-5 minutes, and take the supernatant as the protein extract.

3. Precautions

Steps of protein extraction should be performed on ice or at 4°C. It is recommended to divide the samples into appropriate amounts, and then freeze-dry them or store them in a liquid state at -20°C without repeated freezing and thawing.

2. Protease inhibitors need to be prepared by themselves, and it is recommended to add them within a few minutes before use. PMSF, Cocktail,

Phosphoprotease inhibitor can be ordered from Aspen. Protease inhibitors have high toxicity. For your safety and health, please wear a lab coat and disposable gloves. In case of direct contact, rinse immediately with running water before consulting a healthcare provider.

3. The protein sample obtained by cleavage contains a high concentration of detergent interference, and the Bradford method cannot be used to determine the protein concentration. The BCA protein quantitative kit can be used to determine the protein concentration.
4. Note: A small group of transparent jelly often appears in the lysis product of RIPA lysis solution, which is a normal phenomenon. The transparent gel is a complex containing genomic DNA and the like. In the case of not detecting proteins that bind particularly tightly to genomic DNA, the supernatant can be directly centrifuged to take the supernatant for subsequent experiments; if it is necessary to detect proteins that bind particularly tightly to the genome, the transparent gelatinous gel can be broken up by sonication. Then, the supernatant was collected by centrifugation for subsequent experiments.