Cell lysis buffer for WB and IP without inhibitors

Introduction

Cell lysis buffer for WB and IP without inhibitors is a commonly used rapid lysate for the preparation of protein samples by lysing cell or tissue samples under native conditions. The main components of this product are 20 mM Tris (pH 7.5), 150 mM NaCl, and 1% Triton X-100. Protein samples from WB and IP cell lysates can be used for routine PAGE, western blotting (WB), immunoprecipitation (IP), co-immunoprecipitation (co-IP), and enzyme-linked immunosorbent assay (ELISA). This product can be used on animal and plant cell or tissue samples and can also be used on fungal or bacterial samples.

Protocol

1. Reagents to be prepared by the user

   - Protease inhibitors: PMSF (A2587) or Protease Inhibitor Cocktail (K1007)
   - Phosphatase inhibitors: Phosphatase Inhibitor Cocktail (K1015)
   - Deacetylase inhibitors: Deacetylase Inhibitor Cocktail (K1017)

2. Preparation of lysis buffer

   After thawing Cell lysis buffer for WB and IP without inhibitors, mix it evenly. Take an appropriate amount of the buffer and add the protease inhibitor PMSF to make the final concentration of PMSF reach 1 mM. Appropriate Cocktails of the above-mentioned protease and phosphatase inhibitors can be added according to the experimental requirements.

***Note:** 1. When more comprehensive protection is required, you can choose to replace it with protease inhibitor Cocktail (K1007); 2. If the protected protein is in phosphorylated or acetylated form, the phosphatase inhibitor Cocktail (K1015) or the deacetylase inhibitor Cocktail (K1017) can be used, respectively.

3. Cell or tissue lysis

   3.1 Cell samples

   - Adherent cells

     a. Aspirate the disturbing; wash it with PBS, normal saline, or serum-free culture (if the protein in the serum is not disturbing, it can be left unwashed).

     b. Add lysate in a ratio of 100-200 uL of lysate per well to a 6-well plate. Pipette several times with a

gun to allow the lysate to come into full contact with the cells. Animal cells are generally lysed after 1-2 s of contact with the lysate, while plant cells should be lysed on ice for 2-10 min.

c. After full lysis, the supernatant was extracted by centrifugation at 10,000-14,000 g for 3-5 minutes, followed by PAGE, WB, IP, Co-IP, and ELISA.

- Suspension cells

a. Collect the cells by centrifugation, and gently vortex or flick the bottom of the tube to disperse the cells as much as possible.

b. Add lysate at a ratio of 100–200 uL of lysate per well of cells in a 6-well plate. Gently flick the bottom of the tube to fully lyse the cells. There should be no significant cell pellet after adequate lysis. If the number of cells is large, it is necessary to aliquot into 50-1 million cells/tube before lysing. Large clumps of cells are more difficult to fully lyse, while a small number of cells are relatively easy to fully lyse due to the easy contact between the lysate and the cells.

c. After full lysis, the supernatant was extracted by centrifugation at 10,000-14,000 g for 3-5 minutes, followed by PAGE, WB, IP, Co-IP, and ELISA.

- Bacteria or yeast

a. Take 1 mL of bacterial or yeast solution, centrifuge to remove the supernatant (or wash once with PBS to remove the liquid thoroughly), and gently vortex or flick the bottom of the tube to disperse the bacteria or yeast as much as possible.

b. Add 100-200 uL of lysate, gently vortex or bounce the bottom of the tube to mix and lyse on ice for 2-10 minutes. For better lysis, bacteria and yeast can be digested with lysozyme (BA1242) and wall-breaking enzyme (Lyticase), respectively, before lysis with this lysate.

c. After full lysis, the supernatant was extracted by centrifugation at 10,000-14,000 g for 3-5 minutes, followed by PAGE, WB, IP, Co-IP, and ELISA.

3.2 Tissue samples

a. Cut the tissue into tiny pieces.

b. Add lysate at a ratio of 100-200 uL lysate per 20 mg of tissue. If the lysis is not sufficient, the amount of lysate can be increased appropriately, and if a high-concentration protein sample is required, the amount of lysate can be appropriately reduced.

c. Homogenize with a glass homogenizer until fully lysed or freeze the tissue sample and grind it with liquid nitrogen and add lysate for lysis after sufficient grinding.

d. After full lysis, the supernatant was extracted by centrifugation at 10,000-14,000 g for 3-5 minutes, followed by PAGE, WB, IP, Co-IP, and ELISA.

Note

1. Lysate dosage description: Usually 100 uL of lysate is enough to add 100 uL of lysate to each well of cells or 1 mL of bacterial solution or yeast solution in a 6-well plate, and if the cell density is very high, the dosage of lysate can be appropriately increased to 150 uL or 200 uL. The protein concentration of the supernatant obtained after lysis with 100 uL of this product per 1 million animal cells is about 2-4 mg/mL, which varies from cell to cell.

2. The protein concentration of the supernatant obtained after lysis with 200 uL of this lysate per 20 mg cryopreserved mouse liver tissue was approximately 15-25 mg/mL, which varied from tissue to tissue in different states.

3. If the tissue sample itself is very small, it can be sheared appropriately and directly lysed by adding lysate to make the sample fully lysed by strong vortexing. The supernatant is then also centrifuged for subsequent experiments. The advantage of direct cracking is that it is more convenient and does not need to use homogenizers or grinding equipment, and the disadvantage is that it is not as full of cracking as homogenization or grinding.

4. To achieve the best use effect, it is recommended to use it after appropriate portioning and try to avoid repeated freezing and thawing.

5. This product is for scientific research use only.

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