Cell Lysis Buffer for WB and IP

Introduction

Cell Lysis Buffer for WB and IP is a commonly used rapid lysis reagent 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, 1% Triton X-100, and various inhibitors such as sodium pyrophosphate, β-glycerophosphate, EDTA, Na_3VO_4 and leupeptin, which can effectively inhibit protein degradation and maintain the interaction between original proteins. 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

Required reagents (not provided):

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

1. Lysis buffer preparation

After thawing Cell Lysis Buffer for WB and IP, 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.

2. Cell or tissue lysis

3.1 Cell samples

- Adherent cells

a. Aspirate the culture and 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 lysis buffer in a ratio of 100-200 μL per well to a 6-well plate. Pipette up and down several times to allow the lysis buffer to come into full contact with the cells. Animal cells are generally lysed after 1-2 seconds of contact with the lysis buffer, while plant cells should be lysed on ice for 2-10 minutes.

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 lysis buffer at a ratio of 100–200 μL per well 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 full lysis. If the number of cells is large, it is necessary to aliquot into $5 \times 10^5 – 1 \times 10^6$ cells/tube before lysis. 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 closer 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 μL of lysis buffer, gently vortex or flick 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 Lyticase, respectively, before lysis with the lysis buffer.

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. Dissect the tissue into small pieces.

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

c. Homogenize with a glass homogenizer until fully lysed or freeze the tissue sample and grind it with liquid nitrogen, and add lysis buffer 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. Stored at -20°C for one year.
2. Lysis buffer dosage: 100μL lysis buffer is sufficient for one well of the 6-well plate cell culture, or 1 mL bacteria/yeast culture. If the cell density is very high, the dosage of lysis buffer can be increased to 150 μL or 200 μL accordingly. 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.
3. Using 100 μL lysis buffer, the final protein extract from 10^6 mammalian cell is about 2-4 mg/mL, which may vary in different cell lines.
4. Using 200 μL lysis buffer, the final protein extract from 20 mg cryopreserved mouse liver tissue is about 15-25 mg/mL, the amount may vary depending on different tissues type and conditions.
5. If the tissue sample is very small, dissect it and add to the lysis buffer directly. Vortex vigorously to assist the lysing. Centrifuge and collect the supernatant afterwards. Direct lysis does not require a homogenizer or grinding equipment, but it may not lyse the sample as fully as homogenization or grinding does.In order to achieve the best use effect, it is recommended to use it after appropriate portioning and try to avoid repeated freezing and thawing.
6. Aliquot the product to avoid repeated freeze-thaw cycles.
7. This product is for scientific research use only.

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