Plant RIPA Lysis Buffer (Strong)

Introduction

Plant RIPA Lysis Buffer (Strong) is a commonly used rapid lysis buffer for plant cells, tissues, or protoplasts. Its main components are detergents such as 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS, together with multiple inhibitors including sodium orthovanadate, sodium fluoride, EDTA, and leupeptin, which effectively prevent protein degradation. Protein samples obtained using Plant RIPA Lysis Buffer (Strong) can be used for routine PAGE, Western blotting (WB), immunoprecipitation (IP), and related applications.

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 Plant RIPA Lysis Buffer (Strong), 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 Plant Cell Samples

   a. Harvest plant cells by centrifugation. Add lysis buffer at a ratio of 100-200 μL per 0.5-1 million cells. Pipette up and down several times to fully contact the cells with the lysis buffer, and lie on ice for 2-10 min.

   b. After complete lysis, centrifuge at 10,000-14,000 g for 3-5 min. Collect the supernatant for subsequent PAGE, WB, IP, or other procedures.

3.2 Plant Protoplast Samples

a. Cut tissue into small fragments and prepare protoplasts.

b. Transform protoplasts with plasmids and continue culturing for 16-48 h. Apply appropriate experimental treatments as needed (optional).

c. Harvest protoplasts by low-speed centrifugation at 100-500 g.

d. Add lysis buffer at a ratio of 100-200 μL per 0.5-1 million protoplasts. Gently tap the bottom of the tube to ensure complete lysis. After thorough lysis, no obvious protoplast pellet should remain. If the protoplast amount is large, divide into tubes with 0.5-1 million protoplasts each before lysis. Large clumps of 0.5-1 million protoplasts are more difficult to lyse thoroughly, whereas smaller amounts of 0.5-1 million protoplasts are easier to lyse completely because the lysis buffer readily contacts them.

3.3 Plant Tissue Samples

a. Cut plant tissue into small fragments.

b. Add lysis buffer at a ratio of 100-200 μL per 20 mg of plant tissue. (If lysis is insufficient, the amount of lysis buffer can be increased appropriately. If high-concentration protein samples are required, the amount of lysis buffer can be reduced appropriately)

c. Homogenize with a glass homogenizer until thoroughly lysed. Alternatively, freeze the tissue samples and grind them in liquid nitrogen. After thorough grinding, add lysis buffer for lysis.

d. After complete lysis, centrifuge at 10,000-14,000g for 3-5 min. Collect the supernatant for subsequent PAGE, WB, IP, or other procedures.

Note

1. If the plant tissue samples are very small, they can be cut appropriately and directly lysed by the addition of lysis buffer with vigorous vortexing to ensure complete lysis. Then centrifuge and collect the supernatant for subsequent experiments. Direct lysis is convenient as it does not require a homogenizer or grinding equipment, but the disadvantage is that it may not be as complete as homogenization or grinding.

2. A small piece of transparent gelatinous material often appears in the lysate from Plant RIPA Lysis Buffer. This transparent gel contains genomic DNA and other complexes and is normal. If proteins tightly bound to genomic DNA are not being detected, the supernatant can be directly collected by centrifugation for subsequent experiments. If proteins tightly bound to genomic DNA need to be detected, the transparent gel can be broken up by sonication, followed by centrifugation to collect the supernatant. For the detection of common transcription factors such as NF-κB or p53, sonication is usually not necessary.

3. For best results, it is recommended to aliquot the buffer appropriately to avoid repeated freeze-thaw cycles.

4. All steps for sample lysis should be performed on ice or at 4°C.

5. This product is for scientific research use only.

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