Plant Western Blot and IP Cell Lysis Buffer

P752158

Storage: -20℃. Avoid freeze/thaw cycle.

Introduction:

Plant Western Blot and IP Cell Lysis Buffer employs a non-denaturing lysis method to lyse cells and obtain total proteins. The acquired proteins can be used for PAGE electrophoresis, Western blotting, Immunoprecipitation (IP), co-immunoprecipitation (co-IP), and other experiments. This product is applicable to plant cell, tissue, or protoplast samples, as well as animal cell or tissue samples, fungal, or bacterial samples. Plant cell, tissue, or protoplast samples lysed by this lysis buffer can be used for PAGE, Western blotting, Immunoprecipitation (IP), co-immunoprecipitation (co-IP), ELISA, and related applications.

Plant Western Blot and IP Cell Lysis Buffer mainly consists of Tris, NaCl, low-concentration Triton X-100, low-concentration sodium pyrophosphate, and other components. It also contains a variety of protease inhibitor ingredients, which can effectively inhibit protein degradation and maintain the original protein-protein interactions. The protein obtained using this lysis buffer can have its concentration determined by a BCA protein quantification kit. Due to the presence of interfering substances such as high-concentration Triton X-100, the Bradford method is not suitable for determining the protein concentration of samples obtained from Western Blot and IP Cell Lysis Buffer. This reagent is for research use only and not intended for clinical diagnosis or other purposes.

Operating Procedures (For Reference Only):

a) For Cultured Plant Cell Samples:

1. Collect plant cells by centrifugation. Add the lysis buffer at a ratio of 100–200 μL lysis buffer per 500,000–1,000,000 cells. Pipette gently several times to ensure full contact between the lysis buffer and cells, then lyse on ice for 2–10 minutes.
2. After complete lysis, centrifuge at 10,000–14,000 rpm for 3–5 minutes. Collect the supernatant, which can be used for subsequent experiments such as PAGE, Western blotting, immunoprecipitation (IP), and co-immunoprecipitation (co-IP).

b) For Plant Protoplast Samples:

1. Cut the tissue into small pieces and prepare protoplasts.
2. (Optional) Transform protoplasts with plasmids, culture continuously for 16–48 hours, and treat with appropriate experimental conditions as needed.
3. Collect protoplasts by low-speed centrifugation at 100–500 rpm.
4. Add the lysis buffer at a ratio of 100–200 μL lysis buffer per 500,000–1,000,000 protoplasts. Flick the bottom of the tube gently to mix well and lyse the cells. After complete lysis, there should be no obvious protoplast precipitation. If the amount of protoplasts is large, they must be aliquoted into tubes with 500,000–1,000,000 protoplasts per tube before lysis.

Aggregated protoplasts (500,000–1,000,000) are difficult to lyse completely, while dispersed protoplasts (500,000–1,000,000) are easier to lyse thoroughly because the lysis buffer can make full contact with them.

c) For Plant Tissue Samples:

1. Cut the plant tissue into small pieces.
2. Add the lysis buffer at a ratio of 100–200 μL lysis buffer per 20 mg of plant tissue.
3. Homogenize and grind the tissue with a glass homogenizer until fully lysed. Alternatively, freeze the tissue sample, grind it in liquid nitrogen, and then add the lysis buffer for lysis after sufficient grinding. After complete lysis, centrifuge at 10,000–14,000 rpm for 3–5 minutes. Collect the supernatant, which can be used for subsequent experiments such as PAGE, Western blotting, IP, and co-IP.
4. If the plant tissue sample itself is very small and tender, it can be directly added to the lysis buffer after appropriate cutting, and lysed thoroughly by vigorous vortexing. After complete lysis, centrifuge at 10,000–14,000 rpm for 3–5 minutes. Collect the supernatant for subsequent experiments (PAGE, Western blotting, IP, co-IP, etc.). The advantage of direct lysis is convenience (no homogenizer or grinding equipment is needed), while the disadvantage is that it is less thorough compared to homogenization or grinding.

Precautions:

1. When removing the culture medium from adherent cells, washing is not necessary if proteins in the serum do not cause interference.
2. If lysis is insufficient, the amount of lysis buffer can be appropriately increased; if a high-concentration protein sample is required, the amount of lysis buffer can be appropriately reduced.
3. A small amount of flocculent precipitate may form when this product is stored at low temperatures. It can be dissolved in a water bath, but the dissolution time should be shortened as much as possible to prevent the active components in the lysis buffer from becoming ineffective. After the product is dissolved for the first time, it can be aliquoted into small portions to avoid repeated freeze-thaw cycles.
4. The operation steps for cell lysis should be performed on ice or at 4°C.
5. Please use the reagent as soon as possible after opening to avoid affecting the results of subsequent experiments.
6. For your safety and health, please wear a lab coat and disposable gloves during operation.