CREATIVE BIOMART®
Assay Kit

Plant membrane protein extraction kit

Product Information

Cat.No.

Kit-2166

Product Overview

Plant Membrane Protein Extraction Kit is a high yield membrane protein extraction kit, based on chemical rather than detergent. It can extract membrane proteins from various plants, and can be used for crude protein preparation and membrane protein preparation. The extraction process is simple and convenient. The kit contains a mixture of protease inhibitors and phosphatase inhibitor mixture, preventing the protei degradation by protease, providing a guarantee for extracting high quality protein. Extracted Proteins using this kit has natural activity and can be used for a variety of downstream experiments.

Description

Transmembrane proteins take on various biological functions and play an important role in the occurrence and development of disease. Membrane protein sample preparation needs to take full account of the downstream applications, such as gel analysis and mass spectrometry analysis, so the preparation of membrane protein samples become an insurmountable challenge.
Conventional methods of preparing membrane protein samples are solubilization using detergents and surfactants. Detergent treatment will make the membrane protein loss of its natural structure, thus hindering the functional study of membrane proteins.

Stability

1 year

Storage

Protease inhibitors store at -20°C;
Protein extracts store at 2-8°C.
Protease inhibitors can also be stored at 2-8 °C before being used. Store at -20 °C after opening.

Synonyms

Plant membrane protein; extraction; Plant; Membrane Protein Extraction Kit; Membrane Protein

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Size

50 tests

Kit Components

Extract buffer A: 25ml
Extract buffer B: 250ul
Dilution buffer C: 10ml
Protease inhibitor mixture: 100 ul

Technical Notes

1. The reagents in the cap centrifuge tube should be centrifuged briefly before opening the lid, and the liquid on the inner wall of the cap should be rubbed to the bottom of the tube to avoid liquid spillage when the lid is opened.
2. All reagents in the experiment must be pre-cooled; all appliances must be pre-cooled in a refrigerator at -20 °C. The entire process must keep the sample at a low temperature.
3. The protease inhibitor is solid at 2-8 ° C. When it is taken out from the refrigerator and returned to room temperature or a 37 ° C water bath for a short time, it becomes liquid and needs to be centrifuged to the bottom of the tube to open the lid.
3. If the protease inhibitor solution precipitates during storage, it will not affect the use, and it will be used normally after dissolution.
4. You can add other protease inhibitors according to your own experiment.

Features & Benefits

1. Easy to use.
2. Contain protein stabilizer, ensuring the stability of extracted protein.
3. Background interference is low when detect protein concentration.
4. The protease inhibitor cocktail consists of 6 independent protease inhibitors; each inhibitor specifically inhibits one or more protease activities. The optimized composition of the mixture allows it to inhibit almost all the important protease activities, including serine proteases, cysteine proteases, aspartic proteases, alanyl-aminopeptidases and so on.

Assay Protocol

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1. Extraction solution preparation: Add 2 ul of protease inhibitor mixture per 500 ul of cold Extract buffer A, mix well and place on ice for later use.
□Note□:
a. Prepare the protein extraction solution according to the number of samples to be treated. The protease inhibitor mixture may not be added to the extraction solution at one time.
b. If the extraction solution added with the protease inhibitor is not used within one week, the protease inhibitor needs to be added again before reuse.
c. The protein extraction solution used in the following steps refers to the extraction solution containing the protease inhibitor configured in this step.

2. Take 100-200mg of plant tissue samples after washing and drying and remove leaf stalks and thick veins, and cut them with surgical scissors as much as possible.
3. Add 500ul of Extract buffer A and homogenize well with a homogenizer.
□Note□
a. It can also be added to Extract buffer A after grinding with liquid nitrogen.
b. If there is no liquid nitrogen grinding condition, it can also be directly added to the cold Extract buffer A to be ground on ice.
c. If the tissue sample is very small, it can be cut and added directly to the extract at 2-8 °C for shaking, without homogenization.

4. Transfer the homogenate to another pre-cooled, clean centrifuge tube and shake at 2-8 °C for 1-2 hours.
□Note□
a. This step must be performed at 2-8 °C.
b. Use the lower speed of the oscillator to ensure that the extraction solution can be shaken slightly.
c. If there is no continuous oscillation condition of 2-8 °C, it can be placed directly in the refrigerator at 2-8 °C for 12 hours, and vortexed and mixed every 10 minutes in the middle.

5. Centrifuge the extract solution at 2-8 ° C, 12000 g for 5 minutes, and take the supernatant.
6. Add 5ul of Extract buffer B to the supernatant and mix well.

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7. Water bath at 37 ° C for 10 minutes.
8. Centrifuge at 1000g for 3 minutes at 37 °C.
□Note□
a. This step must be centrifuged at 37 °C.
b. If the centrifuge is not temperature-controllable, do not centrifuge, extend the water bath time of the previous step until the solution is clearly layered. Or centrifuge at room temperature to shorten the centrifugation time to 1 minute.

9. At this point the solution is divided into two layers, carefully removing the upper layer, leaving approximately 30-50 ul of the lower layer of liquid at the bottom of the tube.
□Note□:
a. The lower layer is a viscous liquid.
b. The underlying membrane protein of a chloroplast-containing plant sample is yellow-green and usually does not affect downstream applications.

10. Dissolve the lower layer solution with 50-150 ul of cold Dilution Buffer C to obtain a membrane protein sample.
11. Quantify the above protein extracts, store the spare aliquot in a refrigerator at 80 ° C or directly for downstream experiments.
□Note□:
a. Protein quantification is recommended using the BCA method.
b. Protein samples can be stored at -80 ° C for one year. Be careful not to be hydrolyzed by proteases and not contaminated with bacteria.