Ver. 241001

BC3720

Plant Protein Extraction Kit

ISO 13485:2016 ISO 9001:2015

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(BC3720)

Kit Contents

Contents	50 Preps
Extraction Buffer	150mL
Solubilization Buffer	30mL
Handbook	1

Storage

Plant Protein Extraction Kit could be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer term storage, the kit could be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)

Introduction

Plant Protein Extraction Kit is used to extract total protein from plant tissue. The lysate in the kit contains protease inhibitor, which has mild effect and can quickly obtain total protein. It can be used for basic research experiments such as western blot experiments. This product is used for scientific research only.

Important Notes

1. All centrifugation steps are carried out in refrigerated microcentrifuge at 4℃.
2. The protocol will need standardization depending on the starting material.
3. Add Polyvinylpolypyrrolidone (PVPP) to the Extraction Buffer to a final concentration of 1%.
4. Add β-mercaptoethanol to the Extraction Buffer to a final concentraton of 2%.
5. Prepare only the required volume of Extraction Buffer according to the number of samples. Do not store and use the buffer.

Preparation of Buffers.

Wash Buffer:

- Ammonium Acetate: 0.1M
- Methanol: 100%

Precipitation Buffer:

- Acetone: 100%

Store the buffers at -20℃.

Protocol

Ensure that PVPP and β-mercaptoethanol is added to the Extraction Buffer before use.

1. Flash freeze the tissue in liquid nitrogen and grind 100mg tissue in a pre-chilled mortar and pestle. Transfer the powder to a 2mL microcentrifuge tube.
2. Add 800µL reconstituted Extraction Buffer and use an orbital shaker (150rpm) to homogenize the samples for 30 minutes at 4℃. Keep the tube in the horizontal position for vigorous shaking.
   Note: Homogenization time can be adjusted according to the sample type.
3. Add 800µL equilibrated phenol with 10mM Tris-HCl (pH = 8.0). Homogenize the samples, using an orbital shaker (150rpm), for 30 minutes at 4℃.
   Note: Homogenization time can be adjusted according to the sample type.
4. Centrifuge at 10000×g for 10-15 minutes at 4℃.
5. Recover the supernatant to a new microcentrifuge tube and add 800µL reconstituted Extraction Buffer followed by homegenization using an orbital shaker (150rpm) for 10-15 minutes at 4℃.
6. Repeat steps 4 and 5. Instead of adding Reconstituted Extraction Buffer, add Extraction Buffer without supplementation with PVPP and β-mercaptoethanol.
7. Centrifuge at 10000×g for 10-15 minutes at 4℃.
8. Add 1.6mL of the Wash Buffer and keep the samples at -20℃, overnight.
9. Centrifuge at 10000×g for 10-15 minutes at 4℃.
10. Discard the supernatant and add 1.6mL Wash Buffer without disturbing the pellet, keep the samples at -20℃ for 1 hour.
11. Centrifuge at 10000×g for 10-15 minutes at 4℃.
12. Repeat steps 10 and 11 two more times.
13. Discard the supernatant and add 1.6mL of precipitation buffer without disturbing the pellet. Keep the tube in the freezer (-20℃), for 1 hour.
14. Centrifuge at 10000×g for 10-15 minutes at 4℃.
15. Dry the pellet, seal the tube and store at 4℃, overnight. Dissolve the pellet in 400µL Solubilization Buffer. Quantify the sample and proceed to downstream assays.