

Plant Protoplasmic Preparation Kit

Cat: EX2860

Size: 50T/100T

Validity: 2-8°C storage, valid for one year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Washing solution A	25mL	50mL	2-8°C
Component B: Extract solution B	25mL	50mL	2-8°C
Component C: Extract solution C	250μL	500μL	2-8°C
Component D: Protoplast preservation solution D	25mL	50mL	2-8°C

Note: Please use the reagent as soon as possible after unpacking!

Introduction:

Plant protoplasmic preparation kit provides a complete set of reagents, suitable for preparing protoplasts from various plant samples. This kit provides a system for maintaining protoplasts that are separate and intact. Optimized reagent formulations and procedures allow rapid protoplast separation with minimal cross-contamination.

This kit is suitable for the preparation of protoplasts of various plant samples, and can also be used for the preparation of eukaryotic algal protoplasmic system such as single cell, multicellular, filamentous, multinucleate, etc., such as green algae, chrysoalgae, siloalgae, dinoflagellate and spirulina.

The protoplast components extracted from this kit still maintain their biochemical functions and are suitable for various downstream analysis.

This kit is only suitable for protoplasmic preparation of plant cells during the growth period and those that have just stopped growing. The preparation of protoplasts with very thick secondary walls and those with lignified and silicified secondary walls that have stopped growing for a long time or even died is poor.

Self-prepared reagents and instruments:

Centrifuge, oscillator, vortex mixer, pipette, refrigerator, ice box, PBS buffer, centrifuge tube, suction tip, disposable gloves

Protocols:

First, notes for use:

1. Before the formal experiment, please select several samples to do pre-experiment, in order to optimize the experimental conditions and achieve the best experimental results
2. Centrifuge the reagent in the screw cap microreagent tube briefly before opening the cap, and centrifuge the liquid on the cap and inside wall to the bottom of the tube to avoid reagent loss when opening the cap.
3. All reagents in the process of the experiment must be pre-cooled; All utensils must be

pre-cooled in a -20°C refrigerator. The sample must be kept at a low temperature during the whole process.

Second, plant protoplasmic system preparation:

1. Extraction liquid preparation: Every $500\mu\text{L}$ cold washing solution A, add $5\mu\text{L}$ extract solution C, mix well and reserve.
2. The 100-200mg fresh plant tissue samples, which were washed and dried and the leaves and thick veins were removed, were cut into $0.5\text{mm}\times 0.5\text{mm}$ pieces with A sharp surgical blade, and then thoroughly mixed with $500\mu\text{L}$ of washing solution A.
3. Let the samples stand at 30°C for 15min.
4. Centrifuge at $2000\times g$ for 5min, discard the supernatant and collect the precipitation.
5. Add $500\mu\text{L}$ extract solution B to the precipitation and mix thoroughly.
6. Place the suspension of extract solution B sample in an oscillator at 37°C or room temperature for 24-72 hours.
7. Centrifugal force $1000\times g$ centrifuge for 5min, collect precipitation, that is, protoplast.
8. Wash the protoplasts once with PBS.
9. Add $500\mu\text{L}$ reagent D to the protoplast precipitate to re-suspend the protoplast and mix well.
10. The protoplast samples were stored in the refrigerator at $2-8^{\circ}\text{C}$ for future use or directly used in downstream experiments.

Notes:

1. This kit is intended for scientific research only and is not intended for diagnosis or treatment.
2. It is best to use disposable suction heads, tubes, bottles, or glassware, and reusable glassware must be washed and thoroughly removed of residual cleaners before use.
3. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.
4. Avoid skin or mucous membranes coming into contact with the reagent.
5. If the reagent accidentally comes into contact with skin or eyes, it should be rinsed with water immediately.