Plant Nuclear and Cytoplasm Extraction Kit (Enzymatic method)

Cat: EX2800
Size: 50T/100T
Validity: 2-8℃ storage, valid for one year.

Kit Components:

Kit Components	50T	100T	Storage
Washing solution A	25mL	50mL	2-8℃
Extract solution B	25mL	50mL	2-8℃
Extract solution C	25mL	50mL	2-8℃

Note:

1. Store the kit at 2-8℃, and store the components according to the required conditions after opening the lid.
2. Please use the reagent as soon as possible after unpacking!

Product Introduction:

Plant nucleus and cytoplasm extraction kit provides a full set of reagents, suitable for extracting the nucleus and cytoplasm from various plant cells and various solid plant tissues, such as leaves, roots, seeds and other plant tissues. The extraction process is simple and convenient, and can be completed within 1 hour. The prepared nuclei and cytoplasm are not only high in purity, maintain natural activity, and have little cross-contamination.

This kit uses enzymatic extraction, enzymatic extraction preparation has improved recovery, high purity and natural activity, but it takes a long time. The extraction process of the non-enzymatic extraction kit is simple and convenient, fast, can be completed within 1 hour, and there is little cross contamination, but the recovery rate is lower than that of the enzymatic method. If you need a faster extraction kit, you can choose a non-enzymatic extraction kit, if there is no requirement for extraction speed, you can choose an enzymatic extraction kit. Please select the kit according to your actual needs.

Self-prepared reagents and instruments:

Centrifuge, oscillator, vortex mixer, Dounce homogenizer, pipette, refrigerator, ice box, PBS buffer, centrifuge tube, suction head, cell sieve (100um), disposable gloves

Protocols:

First, notes for use:

1. Please centrifuge the reagent in the rotating cap centrifuge tube briefly before opening the cap, and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid the liquid spilling when opening the cap.
2. All reagents in the experiment must be pre-cooled; All appliances must be pre-cooled in the -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
3. You can add other protease inhibitors according to your own experimental needs.

Second, Plant Nuclear and Cytoplasm Extraction:

1. Take a 100-200mg plant tissue sample that has been washed and dried and has had the leaves and thick veins removed and cut it up as much as possible with surgical scissors (or cut it into as small pieces as possible with a sharp blade).
2. Add 500μL washing solution A and mix well.
3. Let stand at 30°C for 15min.
4. Centrifuge at 2000×g for 10min, discard the supernatant and collect the precipitation.
5. Add 500μL extract solution B to the precipitate and mix well.

[Note]:

① Centrifuge the cultured cells at 1000×g for 5min, carefully absorb the medium, blot as much as possible, wash the cells with PBS twice after collection, and then directly add the extract B for suspension.

② 1mL of extract solution B was added to approximately every 300μL cell volume.

③ Adjust the amount of extraction liquid according to the actual sample volume, the general reagent is 2-3 times the sample volume, and the sample can be fully submerged.

6. The extraction solution B sample suspension is placed on the oscillator 37℃-45℃ or room temperature oscillation 24-72 hours.

[Note]:

① Use the lower speed of the oscillator/shaker, the extraction liquid can be slightly shaken. No oscillating conditions can also not oscillate, in the middle of every few hours with the pipette blow mixing can be.

② Select the appropriate temperature according to the downstream nuclear and cytoplasmic application, and the preparation speed is the fastest when the highest temperature is 55℃. However, too high temperature will affect the sample activity, please choose the temperature according to the downstream application.

③ The processing time of different types of plant samples is very different. Arabidopsis is easier to handle, the fresh leaves are easier to handle, and the processing time of young samples is shorter. Some plant types with thicker cell walls may take longer to handle. Treatment can be extended beyond 72 hours to improve yield.

7. Centrifuge at 2000×g for 5min, discard the supernatant, and collect the precipitation.

[Note]:

① If conditions, it is best to use 100μm cell screen to filter the extract solution B treatment solution, collect the filtrate and then centrifuge.

② No cell screen can not be filtered.

8. Wash and precipitate once with PBS. (Centrifuge at 2000×g for 5min)
9. Add 500μL reagent C to the precipitate and mix thoroughly. Homogenate with a homogenizer/homogenizer.
10. Oscillate at 4 ° C for 20-30min.

① Use the low speed of the oscillator/shaker, the extraction liquid can be slightly shaken.

② No oscillating conditions can also be not oscillating, standing at 2-8℃, slightly extend the processing time, every few hours in the middle with pipette blow mixing can be.

11. Centrifuge at 4℃, 800×g force condition for 5min.
12. Remove the supernatant to another clean centrifuge tube. The supernatant is the cytoplasmic portion and precipitates into the nucleus.
13. The nuclear precipitation is suspended with the corresponding buffer, and the two samples are stored in the refrigerator or directly used for downstream experiments.

Notes:

1. Before the formal experiment, please select several samples for pre-experiment to optimize the experimental conditions and achieve the best experimental results.
2. The reagent in the screw cap trace reagent tube should be centrifuged briefly before opening the cap, and the liquid on the cap and inner wall should be centrifuged to the bottom of the tube to avoid the loss of the reagent when opening the cap.
3. It is prohibited to mix with other brands of reagents, otherwise it will affect the use effect.
4. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may lead to wrong results.
5. It is best to use disposable suction heads, tubes, bottles or glassware, and reusable glassware must be washed and thoroughly removed before use.
6. After the completion of the experiment, all samples and utensils in contact should be disposed of in accordance with the prescribed procedures.