Chloroplast extraction kit

Item No.: EX1390

Specification: 50T/100T

Store: 2-8°C, valid for 1 year.

Product composition:

Product components	50T	100T	Storage conditions
Reagent A: Chloroplast extract A	50mL	100mL	Store at 2-8°
Reagent B: Chloroplast extract B	25mL	50mL	Store at 2-8°
Reagent C: chloroplast preservation solution	20mL	40mL	Store at 2-8°

Note:

1. Use the reagent as soon as possible after unpacking.

Product Introduction:

Chloroplasts are the unique energy conversion organelles of plant cells, and photosynthesis is carried out in chloroplasts. Because of this important function, chloroplasts have been an important object of research in cell biology, genetics and molecular biology.

Chloroplast extraction kit is a simple and rapid method to quickly extract chloroplasts.

This kit is suitable for extracting chloroplasts from fresh plant samples. The chloroplast recovery rate is low because most of the chloroplasts are destroyed in the frozen storage process.

The chloroplasts extracted by this kit have biological activity and can be used in various downstream applications such as chloroplast function research and chloroplast protein extraction.

This kit uses non-enzymatic rapid extraction method, which can quickly extract chloroplasts within one hour, but the recovery rate is slightly lower than that of enzymatic chloroplast extraction kit. If you need an enzymatic extraction kit, you can contact Soleibao to select another kit. The chloroplast recovery obtained by enzymatic extraction will be increased, but it takes a long time. Please select the kit according to your different needs.

Bring your own reagents and equipment:

Centrifuge, oscillator, homogenizer/homogenizer, vortex mixer, pipette, refrigerator, ice box, 1×PBS buffer, centrifuge tube, suction tip, disposable gloves, cell screen (100μm).

Directions to use:

First, precautions for use:

1. Centrifuge speed has relative centrifugal force (RCF, ×g) and speed per minute (RPM) two ways of expression, some centrifuges have RPM and ×g display switching, but some centrifuges do not have automatic switching function. Need to use the following formula for conversion: $g=r\times1.118\times10^{-5}\times rpm^2$ (r is the effective centrifuge radius, the length in centimeters from the centrifuge axis to the center of the bottom of the centrifuge collection tube)

For example, if the rotational speed is 3000rpm and the effective centrifugal radius is 10cm, then the relative centrifugal force (RCF, ×g) is $=10\times1.118\times10^{-5}\times30002=1006.2$ (×g).

Second, operation procedure

1. Take 500mg-1g fresh plant sample leaves, wash and dry them, and remove leaf stems and thick veins. Use surgical scissors to cut up as much as possible.
2. Add 2mL PBS to fully homogenate with a tissue crusher/homogenizer/homogenizer.

[Note] :

1) If there is no homogenizer for leaf and other tissue samples, you can also add a small amount of PBS first and then fully homogenize with Dounce homogenizer and then add PBS to mix.

3. Filter the homogenate through a 100μm cell screen.

[Note] :

1) If there is no cell sieve, let it stand slightly at 4°C, let the coarse fibers and clumps settle, or centrifuge at a

force of less than 100g for 1min, collect the cell supernatant, and discard the tissue precipitation.

2) Some samples containing a lot of mucus may be difficult to absorb, you can slightly cut off the tip of the 1mL suction tip for use.

4. Centrifuge the filtrate at 3000×g for 10min, discard the supernatant, and collect the precipitate.
5. Add 1ml of extract A to the precipitate and mix well.

[Note] :

1) The cultured cells can be centrifuged for 5min at 1000×g, carefully absorb the medium, blot as much as possible, wash the cells with PBS twice after collection, and then directly add the extract A for re-suspension.
2) 1mL of extract solution A was added to approximately every 300μL cell precipitation volume.

6. Fully homogenate with a homogenizer/homogenizer.
7. Centrifuge at 3000×g for 10min, discard the supernatant and collect the precipitate.
8. Add 0.5mL of extract B to the precipitate and mix thoroughly.
9. Set the oscillator to oscillate for 20min.

[Note] :

1) Use the lower speed of the oscillator/shaker, so that the extract can shake slightly.
2) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every fewmin with the pipette blow mixing can be.

10. Centrifuge at 200×g for 2min. Discard the precipitate and remove the supernatant.
11. Centrifuge at 1000×g for 2min. Discard the precipitate and remove the supernatant.
12. Centrifuge the supernatant 3000×g for 10min.
13. Discard the supernatant and precipitate into chloroplasts.
14. The chloroplasts were re-suspended with 400μL chloroplast suspension or other corresponding buffer, stored in refrigerator or directly used for downstream experiments.

Analysis of common problems:

1. What is the purity of the extracted chloroplasts?

The purity of chloroplast samples extracted by this kit is about 90%. It is not recommended for downstream applications such as protein localization.

Note:

1. Before the formal experiment, please select a few samples for 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. Do not 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 result in false results.
5. 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.
6. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.