

# Microalgae Chloroplast Extraction Kit

Cat: EX2820 Size: 50T/100T

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

# **Kit Components:**

Kit Components	50T	100T	Storage
Component A: Microalgae washing solution A	250mL	500mL	2-8°C
Component B: Chloroplast extract solution B	500mL	1000mL	2-8°C
Component C: Chloroplast extract solution C	250mL	500mL	2-8°C
Component D: Chloroplast preservation solution D	100mL	200mL	2-8°C

#### Note:

- 1. It can also be stored at 2-8°C before use without open cover, and it is not needed to be stored at -20°C for a long time.
- 2. Please use the reagent as soon as possible after unpacking!

#### Introduction:

Chloroplast is the unique energy conversion organelle of plant cells, photosynthesis is carried out in chloroplast, because of this important function, so chloroplast has been an important research object in cell biology, genetics and molecular biology.

Chloroplast extraction kit of microalgae can be used to quickly extract chloroplasts from various microalgae by simple and rapid method.

# Self-prepared reagents and instruments:

Centrifuge, ultrasonic cell crusher, vortex mixer, pipette, refrigerator, ice box, PBS buffer, protein quantification kit, centrifuge tube, suction tip, disposable gloves, cell screen (40µm/70µm)

#### **Protocols:**

# First, notes for use:

The following part of the operation steps for different types of microalgae samples need to adjust the operating conditions according to the pre-experiment situation.

## Second, Microalgae Chloroplast Extraction:

- 1. Take a number of microalgae in the middle of growth and place them in a refrigerator at 4°C away from light for 24-36h.
- 2. Centrifuge at 2000×g for 5min, remove the supernatant, and collect the precipitated microalgae.
- 3. Wash the precipitated microalgae once with PBS. (2000×g centrifuge for 5min)
- 4. Add 10mL microalgae washing solution A into 3-5g microalgae cell precipitation and mix thoroughly.
- 5. Let stand at room temperature for 2-3min.
- 6. Centrifuge at 2000×g for 10min, remove the supernatant, and collect the precipitated microalgae. (Blot as much supernatant as possible)
- 7. Add 10mL of microalgae extract solution B to the precipitate and mix thoroughly.
- 8. Extract solution B at 300w, ultrasonic 3s, interval 3s condition ultrasonic treatment 1-15min.

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## [Note]:

- ① The treatment time of different algae varies greatly, and the ultrasonic time can be adjusted according to the pre-experiment results. In general, chlamydomonas and chlorella were treated for 5-8min, chrysochlorella 2-3min, and flagellate, dinoflagellate and spheroflagellate were treated for 1min.
- ② Without ultrasonic conditions, the extracted liquid microalgae suspension can be added to the mortar and a small amount of quartz sand is added for grinding.
  - ③ Cells can also be broken with a high pressure cell crusher.
- 9. Filter the extract B through a 40µm cell sieve. (No cell sieve can be left unfiltered)
- 10. Centrifuge the filtrate at 800×g for 3min, discard the precipitation, and retain the supernatant.
- 11. Centrifuge the supernatant at 3000×g for 10min, discard the supernatant, and collect the precipitation.
- 12. Re-suspension precipitation with 5mL reagent C.
- 13. Centrifuge at 3000×g for 10min, discard the supernatant, and collect the precipitation.
- 14. Discard the supernatant and the precipitation is chloroplast.
- 15. The chloroplasts were re-suspended with 2ml chloroplast preservation solution or other corresponding buffer solution, stored in the refrigerator or directly used in downstream experiments. It is also possible to use chloroplasts directly for downstream experiments or preserve chloroplasts with your own buffer according to the needs of downstream experiments without using the chloroplast preservation solution in the kit.

#### 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.