

INTENDED USE

AZUL Chloroplast DNA Extraction Kit offers a simple and effective method for isolating total DNA from intact chloroplasts in plant leaves.

SUMMARY AND EXPLANATION

This kit uses a silica-based spin column technology for isolating DNA from biological samples, thereby eliminating toxic phenol-chloroform extractions. The eluted DNA is suitable for all sensitive downstream applications such as qPCR and Next-Generation sequencing.

PRODUCT FEATURES

- Rapid purification of high-quality, ready-to-use DNA.
- No organic extraction or alcohol precipitation.
- Consistent and high yields.
- Complete removal of contaminants and inhibitors for reliable results.
- Kit formats for low- to high-throughput – options for automation of all kits.

PRECAUTIONS

- Avoid all skin contact with reagents in this kit. In case of contact, wash thoroughly with water.
- AZUL Chloroplast DNA Extraction Kit is intended for use as supplied. Do not dilute or add other components to the AZUL Chloroplast DNA Extraction Kit.

DIRECTIONS FOR USE

Chloroplast Isolation:

1. Prior to extraction, about 5 g (fresh weight) leaves were collected and kept in dark for 48 to 72 hours at 4°C in order to decrease starch level stored in the leaves.
2. The leaves were cut into pieces (1 cm) and homogenized in 25ml ice-cold isolation buffer for 30 seconds.
3. Filter the homogenate into centrifuge tubes using two layers of miracloth/muslin with softly squeezing the cloth.
4. Centrifuge the homogenate at 3000 rpm for 10 mins.
5. Resuspend the chloroplast pellet in 5mL cold isolation buffer, and re-pellet the chloroplasts by centrifuging at 3000 rpm, for 10 mins.
6. Resuspend the final chloroplast pellet in 0.5mL cold isolation buffer and transfer to a 2mL tube for further DNA isolation from the obtained chloroplasts.
7. Take 5 µL of the suspension and place it onto a clean microscope slide. Gently position a cover slip over the sample. Observe under a microscope using the 40x objective lens, to check for the presence of chloroplasts, before proceeding with DNA isolation.

Chloroplast DNA Extraction:

1. Add 1mL of CP-Extraction Buffer to the pellet suspended in cold isolation buffer and 25µL CP-lysis buffer, vortex briefly. Add 20µL of Proteinase K, mix briefly by inverting tubes and Incubate at 55°C for 1 hour.
2. Centrifuge the tubes at 12,000 rpm for 15 mins.
3. Transfer the clear supernatant to a fresh microfuge tube and add 600µL of Binding Buffer, mix well by inverting the tubes and incubate at -20°C for 15 mins.
4. Transfer the suspension to a spin column and centrifuge the tube at 12,000 rpm for 2 min at RT.
5. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until complete lysate has been transferred into the column and centrifuged.
6. Wash the spin column with 500µL Wash Buffer (WB) at 12,000 rpm for 1 min and discard the flow through. Repeat this step again.

7. Keep the purification column in a clean, sterile 1.5 mL microfuge tube and add 30µL- 50µL of Elution Buffer or DNase/RNase-free water to the center of the column.
8. Centrifuge the column for 12,000 rpm for 2 min.
9. Discard the purification column and store the eluted DNA at -20°C or -80°C until use.

KIT COMPONENTS

Components	For 50 preps	For 25 preps
Cold Isolation Buffer	1800mL	900mL
CP-Extraction Buffer	50mL	25mL
CP- Lysis Buffer	1.5mL	0.7mL
Proteinase K	1mL	0.5mL
Binding buffer(BB)	30mL	15mL
Wash Buffer (WB)	60mL	30mL
Elution Buffer(EB)	4mL	2mL
Spin Column	50 (Pouch pack)	25 (Pouch pack)

CAUTION

- Check the buffers for any salt precipitation before every use.
- Re-dissolve any precipitate by warming the solution to 37°C, then cool it back to room temperature before use.
- During operation, always wear a lab coat, disposable gloves, protective goggles and mask.

KIT STORAGE AND STABILITY

- Store the kit at room temperature.
- Viable for 1 year if stored at appropriate conditions.

ORDERING INFORMATION

Please call us at +91 8088747968 or mail at [hello@azooka.life](mailto:hello@azooka.life) for any queries or assistance. Additional information can be found online at [www.azooka.life](http://www.azooka.life)