CE IVDR

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AZUL CHLOROPLAST DNA EXTRACTION KIT

GOOD YIELDS FOR USE IN PCR/SEQUENCING

PRODUCT BROCHURE

Cat No-DE116

ISO 13485 CERTIFIED

PRODUCT DESCRIPTION

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

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)

SPECIFICATIONS

Format	Spin column
Sample type	Plant tissues like leaves
Equipment	Microcentrifuge
Processing time	120 mins
Sample amount	≥ 5 g
Type	Total cpDNA
Sample storage	Eluted DNA should be stored at ≤ -20°C
Yield	2-10 µg
Purity	A260/280 ≥ 1.8
Kit Storage	2-8°C Proteinase K at -20°C
Kit Validity	Viable for 1 year if stored at appropriate conditions

NOTE: *Check the Extraction Buffer and Binding Buffer 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.*

BUFFER PREPARATION

- Dissolve the given BSA in 10 mL of Milli Q /Nuclease Free water and store at 2-8°C. Add 4.5 mL of dissolved BSA to each 450mL Cold isolation buffer before DNA extraction.
- Add 0.5mL of the given Beta-Mercaptoethanol to each 450mL Cold isolation buffer before DNA extraction.
- After adding BSA and β-Mercaptoethanol, thoroughly mix the buffer before extraction, and ensure it is always stored and used under cold conditions.
- Add 0.5mL of Milli Q /Nuclease Free water to Proteinase K, dissolve completely and store at -20°C.

DNA EXTRACTION PROTOCOL

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.

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DNA EXTRACTION PROTOCOL

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.

- *Avoid harsh or prolonged homogenization, as this may prevent the isolation of intact chloroplasts.*
- *Homogenize just until the lysate turns green to dark green.*
- *To reduce cellular debris, after filtering through Miracloth or muslin cloth, pass the filtrate through Whatman filter paper (pore size 24 cm) before proceeding with centrifugation.*
- *Use ice-cold binding buffer to enhance binding efficiency.*
- *On addition of elution buffer to the column incubate at RT for 2 mins, for efficient elution.*

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FLOW DIAGRAM OF DNA EXTRACTION PROTOCOL

Weigh 5 g of plant tissue, cut into pieces

Add 25 ml ice-cold Cold isolation buffer and homogenize

Filter the homogenate

Centrifuge at 3000 rpm for 10 mins

Resuspend the chloroplast pellet in 0.5mL ice-cold Cold isolation buffer

Centrifuge at 3000 rpm for 10 mins

Isolated chloroplasts

Add 1mL of CP Extraction Buffer + 25 µL CP lysis buffer + 20 µL Proteinase K

Incubate at 55°C for 1 hour

Centrifuge at 12,000 rpm for 15 mins

Add 600 µL Binding Buffer

Keep at -20°C for 15 mins

Mix well
Transfer lysate to Spin column

Centrifuge at 12,000 rpm for 2 mins

Add 500 µL wash buffer

Centrifuge at 12,000 rpm for 1 min

Repeat this step

Add 30-50 µL elution buffer

Centrifuge at 12,000 rpm for 2 mins

Eluted DNA

Store at -20°C or -80°C until use

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSES	SUGGESTED SOLUTIONS
Low DNA Yield	**Tissue input:** Too much input or significantly less tissue used	Use less input material or increase the volume of the Lysis Buffer and lyse organelles thoroughly. Use of ≥ 5 mg is recommended for good DNA yield.
	Incomplete Debris Removal or incomplete lysis/homogenisation can cause cellular debris to clog or overload the column and leech salts into DNA eluate.	Increase the volume of Extraction Buffer to ensure complete lysis/ homogenization. Be sure to centrifuge and pellet any cellular debris and transfer the supernatant while avoiding any pellet debris.
Low DNA Purity(A260/A280)	Improper sample handling results in ethanol or salt contamination	Make sure lysate and wash buffers have passed entirely through the matrix of the column. This may require centrifuging at a higher speed or longer time.
RNA Contamination	Too much tissue used	**To remove RNA:** Perform in-column RNase I treatment or perform RNase I treatment post-purification (not provided in the kit), then re-purify the treated sample.
DNA Degradation	Use of old tissue samples	**To prevent DNA degradation:** Immediately collect and lyse fresh samples into a isolation Buffer.

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ORDERING INFO

CATALOG NO	PRODUCT	PREP
DE101	AZUL Tissue DNA Extraction Kit	25/50 preps
DE102	AZUL Animal Cell Culture DNA Extraction Kit	25/50 preps
DE103	AZUL Bacterial DNA Extraction Kit	25/50 preps
DE104	AZUL Plasmid DNA Extraction Kit	25/50 preps
DE105	AZUL Plant DNA Extraction Kit	25/50 preps
DE106	AZUL Soil DNA Extraction Kit	25/50 preps
DE107	AZUL Blood DNA Extraction Kit	25/50 preps
DE108	AZUL Cell-free DNA Extraction Kit	25/50 preps
DE109	AZUL DNA Extraction Kit- Difficult samples	25/50 preps
DE110	AZUL Saliva DNA Extraction Kit	25/50 preps
DE111	AZUL Stool DNA Extraction Kit	25/50 preps
DE112	Quick AZUL Bacterial/Fungal DNA Extraction Kit	25/50 preps
DE113	AZUL Microbiome DNA Extraction Kit	25/50 preps
DE114	AZUL Gel DNA Extraction Kit	25/50 preps
DE115	AZUL FFPE DNA Extraction Kit	25/50 preps
DE116	AZUL Chloroplast DNA Extraction Kit	25/50 preps
DE117	AZUL Mitochondrial DNA Extraction Kit	25/50 preps
DE118	AZUL Pollen DNA Extraction Kit	25/50 preps
DE119	AZUL Fungal DNA Extraction Kit	25/50 preps
DE120	AZUL Sperm DNA Extraction Kit	25/50 preps
DE121	AZUL Skin DNA Extraction Kit	25/50 preps

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FEEDBACK

How did this kit perform?

Did AZUL Extraction Kit fulfill expectations required for your research?
Let us know by filling out the feedback form here

Or scan the QR code:

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