

Plant DNA Isolation Kit

Cat. No.: S-1030-5; 5 applications

Cat. No.: S-1030; 25 applications

Cat. No.: S-1030-1; 50 applications



MANUAL

Description: DENAZIST Plant DNA Isolation kit is designed for quick and easy extraction of genomic DNA from plant tissues. Using this kit it would be possible to extract genomic DNA with the highest quality. The extracted DNA could be used in a multitude of downstream procedures including PCR, genotyping, sequencing, and different hybridization techniques including Southern blotting.

VERSION: April 2024

Important instructions before first use

For 5 application kit, add 0.88 ml isopropanol to DG2 and 2.4 ml ethanol (96%-100%) to DG3 containers.

For 25 application kit, add 4.4 ml isopropanol to DG2 and 12 ml ethanol (96%-100%) to DG3 containers.

For 50 application kit, add 8.8 ml isopropanol to DG2 and 24 ml ethanol (96%-100%) to DG3 containers.

Label the containers to record that isopropanol and ethanol have been added before first use.

PROTOCOL for isolation of Genomic DNA from Plant

Before the first use, make sure that isopropanol and ethanol have been added to the containers labeled DG2 and DG3, respectively.

-----PREPARATION & DNA RELEASE -----

- Grind 20 mg solid tissue sample in liquid nitrogen in a mortar and pestle. Add 10 mg of ground tissue to a 1.5 ml microfuge (Eppendorf) tube.
- Add 500 µl of DG1 buffer and 5 µl 2-mercaptoethanol to this microtube and mix well. By vortexing and pipetting, immediately resuspend the tissue lysate in the solution.
- Homogenize the tissue/cell lysate by passing it through a 20-gauge needle fitted to a sterile syringe at least 5 times. Transfer the homogenized lysate into a 1.5 ml microfuge tube. Place the tube in the -80 °C (or -20 °C) freezer for 15 min. Then, to thaw the frozen lysate, leave the tube at room temperature.

Extra steps for plants with high levels of polysaccharides

- After finishing step 3, add another 500 µl of DG1 buffer and mix. Add 225 µl of 2 M potassium acetate (pH 5.5; not provided in the kit).
- Mix by inversion. Incubate on ice for 15 minutes. Centrifuge the tube at 10,000 rpm for 10 min at room temperature.
- Transfer the supernatant into a new tube. Add equal to half the volume of the whole mix from 96-100% ethanol. Mix the contents by pipetting.
- Follow the protocol from step 5.

-----DNA BINDING TO THE COLUMN-----

- Centrifuge the tube at 10,000 rpm for 2 minutes. Transfer the supernatant into a new tube. Add equal to the half volume of the lysate from 96-100% ethanol. Mix the contents by pipetting.
- Transfer 700 µl of the mix + ethanol into a new spin column inserted into a collecting tube (available in the kit).
- Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through accumulated in the

collection tube. Remount the spin column onto the collection tube. Transfer the rest of the lysate into the same tube by repeating steps 6 and 7.

-----WASHING-----

- Add 500 µl from the **DG2** solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
- Add 700 µl from the **DG3** solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
- Without adding any solutions, spin the tube one more time at top speed (13,000 rpm) for 3 min.

-----ELUTION-----

- Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge tube.
- Add 50-100 µl from the **DG4** solution onto the center of the spin column. Leave the spin column mounted on the microfuge tube for 5 min at room temperature. Centrifuge the spin column mounted on the microfuge tube at 13,000 rpm for 1 min.
- The eluted solution at the bottom of the microfuge tube contains pure genomic DNA. Until further downstream processes store the sample in -20 °C freezer. ▲

Kit components:

	5 app.	25 app.	50 app.
DG1	5 ml	25 ml	50 ml
DG2	3 ml	15 ml	30 ml
DG3	1.8 ml	9 ml	18 ml
DG4	1 ml	3 ml	6 ml
Spin columns	5	25	50

Store all components at room temperature.

Expiry date: 18 month from the date of manufacture

Date of manufacture:

Notes: Research Use Only
This product insert declares that this products has been analyzed and passed the quality control tests at the time of manufacture.