

Fungi DNA Isolation Kit

Cat. No.: S-1034-5; 5 applications
Cat. No.: S-1034; 25 applications
Cat. No.: S-1034-1; 50 applications



Expiry date: 18 month after shipping

MANUAL

Description: DENAZIST Fungi DNA Isolation Kit is designed for easy extraction of genomic DNA from filamentous fungi, plant tissues, and seeds. The extracted DNA could be used in a multitude of downstream procedures including PCR, genotyping, DNA digestion, and sequencing.

VERSION: Sept 2023

Important instructions before first use

For 5 application kit, add 3.6 ml ethanol (96%-100%) to FG3 container. For 25 application kit, add 18 ml ethanol (96%-100%) to FG3 container. For 50 application kit, add 36 ml ethanol to FG3 container.

Label the container to record that ethanol has been added before first use.

PROTOCOL for isolation of Genomic DNA from Fungi

Before the first use, make sure that ethanol has been added to the container labeled FG3.

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

1. Add 500 μ l of **FG1** buffer to a microcentrifuge tube. Add 5 μ l from 2-mercaptoethanol (not provided in the kit) and mix.
2. Weigh 100 mg fungi/plant tissues/seeds. Add liquid nitrogen and immediately after evaporation grind the sample in a mortar and pestle. Collect the ground sample from the mortar by adding 500 μ l of mix from step 1 and transfer the mix into a microcentrifuge tube.

OR

When you start from a fungal culture, add 1800 μ l of the culture (1×10^8 fungal cells) to a 2 ml microcentrifuge tube. Centrifuge for 3 min at 13000 rpm. Remove all of the supernatant with a pipette tip. Add 500 μ l of the mix from step 1 and resuspend the pellet of cells.

3. Add glass beads and vortex for 10 minutes at maximum speed.
4. Add 10 μ l **proteinase K**, vortex for 10 seconds, and incubate the tube at 60 $^{\circ}$ C for 30-60 minutes.
5. Centrifuge the tube for 5 min at 13,000 rpm. Transfer 500 μ l from the supernatant to a new tube. Discard the pellet.
6. Add 10 μ l of **RNase A** to the lysate, mix by pipetting, and incubate for 30 minutes at 37 $^{\circ}$ C.
7. Add 500 μ l **FG2** buffer. Mix the contents by pipetting. Add 500 μ l ethanol. Vortex for 5 seconds.

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

8. Transfer 700 μ l of lysate into a spin column (inserted into a collection tube, both provided in the kit). Centrifuge the spin column at 8,000 rpm for 1 min. Discard the flow-through accumulated in the collection tube. Remount the spin column onto the collection tube.
9. Transfer rest of the lysate into the spin column and repeat step 8.

-----WASHING-----

10. Add 700 μ l from **FG3** solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
11. Add 500 μ l from **FG3** solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
12. Without adding any solutions, spin the tube one more time at top speed (13,000 rpm) for 5 minutes.
13. Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge (Eppendorf) tube.

-----ELUTION-----

14. Add 50-100 μ l from **FG4** solution onto the center of spin column. Leave the spin column mounted on the microfuge tube for 5 min at room temperature. Centrifuge the spin column mounted on microfuge tube at 10,000 rpm for 1 min.
15. Return the eluted solution from the previous step back onto the center of spin column. Leave the spin column mounted on the microfuge tube for 3 min at room temperature. Centrifuge the spin column mounted on microfuge tube at 13,000 rpm for 2 min.
16. The eluted solution at the bottom of microfuge tube contains genomic DNA. Until further downstream processes store the sample in -20 $^{\circ}$ C freezer. ▲

Kit components:

	5 app.	25 app.	50 app.
FG1	3 ml	15 ml	30 ml
FG2	3 ml	15 ml	30 ml
FG3	2.7 ml	13.5 ml	27 ml
FG4	1 ml	3 ml	6 ml
Proteinase K	55 μ l	275 μ l	550 μ l
RNase A	55 μ l	275 μ l	550 μ l
Glass beads	✓	✓	✓
Spin columns	5	25	50

Store all components at room temperature except the enzymes which should be stored at -20 $^{\circ}$ C.

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.