Rapid Fungal Genomic DNA Extraction Kit

Code No.： NG1002S 100T

NG1002M 200T

Contents	storage	100 T	200T
RNase A (10 mg/ml)	-20 ℃	400 μl	800 μl
Buffer PHY	RT	50 ml	100 ml
Buffer P1	-20 ℃	60 ml	120 ml
Buffer P2	-20 ℃	14 ml	28 ml
Buffer P3	-20 ℃	100 ml	200 ml
Buffer WB	RT	25 ml	50 ml
		Add absolute alcohol before use	
Buffer EB	RT	20 ml	40 ml
Filter Columns AF/ Collection tubes	RT	100	200
Spin Columns AC/ Collection tubes	RT	100	200

Important Note：

All solutions should be clear. If the ambient temperature is low, the solutions may form precipitates and should not be used directly. In such cases, they be heated in a 65 ℃ water bath for a few minutes to restore clarity. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure the reagents to air, the caps of each solution should be tightly closed immediately after use.

Introduction：

This kit utilizes a DNA adsorption column and a novel, unique solution system, making it suitable for the rapid and simple extraction of genomic DNA from fungal tissue. The purification of one or more 100 mg fresh or 20 mg dried fungal samples can be completed within 30 minutes. The extraction process does not require organic solvents such as phenol-chloroform, nor does it require time-consuming isopropanol or ethanol precipitation. It can rapidly and efficiently remove impurities such as polysaccharides, phenols, enzyme inhibitors. The purified DNA can be directly used for experiments such as PCR, restriction enzyme digestion, and hybridization.

A unique lysis buffer rapidly lyses cells, DNA is adjusted for binding conditions, it is selectively adsorbed onto silica matrix membrane in the spin column under high-chaotropic salt conditions. Then, through a series of rapid wash-and-centrifugation steps, impurities such as cellular and proteins are removed. Finally, low-salt water elutes the purified DNA from the silica matrix membrane.

Safety Information:

1. Lysis buffer P1 and P3 contain irritating and harmful compounds. Wear latex gloves during operation to avoid contact with skin, eyes and clothing. If contact with skin or eyes occurs, rinse with plenty of water or saline.
2. Simple and fast, single sample operation can generally be completed within 1 h, the simplest and fastest kit in the world.
3. Multiple rinsing steps to remove proteins result in higher DNA purity.
4. Pre-warm the required water bath to 65 ℃ before starting the experiment.
5. All centrifugation steps are performed at room temperature, and the centrifuge speed must reach 13,000 rpm.

Operation Procedure:

Tip: Before the first use, please add absolute alcohol in the buffer WB and mark it.

Column equilibration step: Add 500 µl of equilibration buffer to the adsorption column AC (place the adsorption column into the collection tube), centrifuge at12,000 rpm for 1 minute, discard the filtrate in the collection tube, and place the adsorption column back into the collection tube. (Please use columns processed the same day. Column equilibration helps to increase the nucleic acid yield.)

Note: Pre-warm buffer P1 at 65 ℃ before use.

Take an appropriate amount of fungal tissue, add liquid nitrogen in a mortar, grind thoroughly into a fine powder.

1. Prepare a 1.5 ml centrifuge tube, add 550 µl buffer P1 and 4 µl of RNase (10 mg/ml), and set aside at room temperature.

   Optional: When the polysaccharide content is particularly high, 2% PVP40 can be added to P1; when the polyphenol content is particularly high, 0.2% 2-mercaptoethanol can be added to P1. Both can also be added simultaneously.
2. Grind fresh or -70 ℃ frozen materials (100 mg of fresh tissue or 20 mg of dry weight tissue (a slightly larger amount of sample can be taken to compensate for the loss adhering to mortar) into a fine powder in liquid nitrogen.
3. Transfer fine powder ((100 mg of fresh tissue or 20 mg of dry weight tissue) into a prepared tube of P1. Immediately vortex or mix by pip up and down to lyse the sample, then place back into a 65 ℃ water bath a short time (5-10 min), occasionally inverting 1-2 times in between to aid lysis.

   Note: This step can also be performed at room temperature by incubating for 10 minutes, but the DNA yield will be slightly reduced.
4. Add 130 µl buffer P2, mix thoroughly, ice bath 5 min. Centrifuge at 12,000 rpm for 5-10 min. Pipette the aqueous phase out into a new 1.5 centrifuge tube. Pipette the aqueous phase out into Spin Columns AF, centrifuge at 12,000 rpm for 2 minutes, and transfer the filtrate collected in the collection tube to a new 1.5 centrifuge tube.
5. Add 1.5 volume P3. Immediately pipette up and down to mix, do not centrifuge. Transfer the resulting solution and any possible precipitate into a adsorption column AC.
6. Centrifuge for 30-60 sec at 12,000 rpm. Discard the flow-through and set the Spin Column AC back into the Collection Tube.
7. Add 600 µl of Buffer WB to the Spin Column and centrifuge for 30 sec, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
8. Repeat step 7.
9. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
10. Add 30-100 µl of Buffer EB or water to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min at 12,000 rpm (The elution buffer can be preheated at 65-70 ℃ to increase the elution effect).

    Note: The volume of elution buffer should not be less than 30 µl, otherwise it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting.
11. DNA can be stored at 2-8 ℃, and for long-term storage, it can be kept at -20 ℃