DNA extraction kit for trace clinical samples

Code No.：NG1005S 50 T

Contents	storage	50 T
Acryl Carrier	-20 ℃	200 μl
Proteinase K	-20 ℃	20 mg
Buffer PHY	RT	5 ml
Buffer ML	RT	20 ml
Buffer CB	RT	20ml
Buffer IR	RT	25 ml
Buffer WB	RT	15 ml Add absolute alcohol before use
Buffer EB	RT	15 ml
Spin Columns DC/ Collection tubes	RT	50

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 37 ℃ 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 CB and IR 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 20 min, the simplest and fastest kit in the world.
3. Multiple rinsing steps to remove proteins result in higher DNA purity.
4. All centrifugation steps are performed at room temperature, and the centrifuge speed must reach 13,000 rpm.
5. To avoid loss of activity and facilitate transportation, Proteinase K is provided as a lyophilized powder. Upon receipt, it can be briefly centrifuged and dissolved by adding 1 mL of sterile water. Since repeated freeze-thaw cycles may reduce enzyme activity, it should be immediately aliquoted according to the amount used each time

and at -20 ℃ after dissolution.

6. Equipped with Acryl Carrier to fully collect extremely trace amounts of DNA.

NOTE:

How to use Buffer PHY:

It can be improved the binding ability of nucleic acid of silica gel column, which is pretreated with buffer PHY. Buffer PHY is a strong alkaline solution, Be careful with it. Store at room temperature. There may be precipitate formation during storage, please heat to 37 ℃ to make the precipitate completely disappear.

Usage: take a new spin column in the collection tube, add 100 µl of buffer PHY to the column, 13,000 prm centrifuge for 1 min, Discard the flow-through and place the spin column back in the same collection tube, follow the next step.

How to use Acryl Carrier:

If the starting sample amount is very small (few cells collected on the oropharyngeal swab), we using Acryl Carrier. If a relatively large DNA yield is expected, users can choose whether to add Acryl Carrier as needed.

When using, add 4 µl of Acryl Carrier to the 400 µl of Binding Buffer CB required for each sample extraction, and thoroughly mix the Binding Buffer CB and Acryl Carrier solution by inverting (Binding CB foams easily, please do not use a vortex mixer to mix). Alternatively, depending on the number of samples, you can add the total required amount of Acryl Carrier to the required amount of Binding Buffer CB and mix it for later use. The mixture is stable at room temperature for 24 hours.

Operation Procedure:

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

1. Blood sample: take 1-100 µl of whole blood;
   Dried blood spot: use a hole puncher to punch out 3 (1/8 inch) diameter blood card discs (containing dried blood spots) from the blood card, up to 3 discs of 3 mm diameter;
   Tissue sample: <10 mg of fresh or thawed tissue after grinding it into a fine powder in liquid nitrogen or cutting it into small pieces with a scalpel (cutting into micro-pieces can the yield).
2. Add a trace amount of sample (ground into powder or liquid) to 400 µl lysis buffer ML.
3. Add 20 µl Proteinase K (20 mg/ml), immediately pipette up and down to mix, 56 ℃ for 1 h, vortex mix for 10 sec every 10 min during the process.
4. 400 µl buffer CB (add 4 µl Acryl Carrier), immediately pipette up and down to mix, 70 ℃ for 10 min.
5. Cool to room temperature, add 200 µl ethanol. Immediately pipette up and down to mix, do not centrifuge. Transfer the resulting solution and any possible precipitate into a adsorption column DC.
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 500 µl of Buffer IR 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. 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.
9. Repeat step 8.
10. Centrifuge for an additional 2 min, 12,000 rpm. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
11. Add 20-50 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 20 µl, otherwise it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting.
12. DNA can be stored at 2-8 ℃, and for long-term storage, it can be kept at -20 ℃