

blood DNA Extraction Kit

Code No. : NG404S 100 T
NG404M 200 T

Contents	storage	100 T	200 T
Buffer PHY	RT	10 ml	20 ml
Buffer PBS	RT	30 ml	60 ml
Buffer CB	RT	30 ml	60 ml
Buffer WB	RT	25 ml Add absolute alcohol before use	50 ml
Buffer IR	RT	50 ml	100 ml
Protease K	-20 °C	20 mg*2	20 mg*4
Spin Columns AC	RT	100	200
Buffer EB	RT	15 ml	30 ml
Collection tubes (2ml)	RT	100	200

Storage:

1. All solutions should be clear, but it may form precipitates if temperature is low, can be heated in a 37 °C water bath for a few minutes to restore clarity.
2. Avoid reagent exposure to the air for a long time, which could be volatilization, oxidation, pH changes, the solution should be timely close the lid after use.

Introduction :

A unique combination of lysis buffer/proteinase K rapidly lyses cells and inactivates intracellular nucleases, then genomic DNA is selectively adsorbed on a silica matrix membrane in a centrifuge column, followed by a series of rapid rinsing-centrifugation steps, the primers, nucleotides, proteins, enzymes and other impurities were removed by rinsing solution. Finally, the pure DNA was eluted from the silicon matrix membrane by low salt and high pH elution buffer solution.

Product and Safety Specifications :

1. The adsorption amount among the columns is very small, and the repeatability is good.
2. The kits provide high yields of pure nucleic acids in minimal elution volumes. The highly concentrated DNA is suitable for direct use in applications, such as: enzyme digestion, ligation and transformation et al.
3. All centrifugation steps are performed at room temperature, and the centrifuge speed must reach 13,000 rpm.
4. The improved solution formulation greatly improves the buffer capacity and stability.
5. 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.
6. Multiple column washes ensure high purity, with a typical yield of 3-12 µg from 200 µl of whole blood, typical OD260/OD280 ratio of 1.7-1.9, and lengths up to 30 kb - 50 kb, which be directly used for PCR, Southern blot, and various restriction enzyme reactions.

NOTE:

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 °C 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 rpm centrifuge for 1 min, Discard the flow-through and place the spin column back in the same collection tube, follow the next step.

Operation Procedure:

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

1. Take 200 μ l of fresh, frozen, or anticoagulated blood and place it into a 1.5 ml centrifuge tube (For large volumes of blood, select code No.405S or 406S). If the initial volume of whole blood is less than 200 μ l, make up to 200 μ l with ddH₂O. If the initial volume is between 200 μ l and 300 μ l, the subsequent steps require a proportional increase in volume.
If the blood sample being processed is anticoagulated blood from poultry, birds, amphibians, or lower organisms, their erythrocytes are nucleated cells. Therefore, the processing volume is 5-20 μ l, which can be made up to 200 μ l with PBS buffer before proceeding to the subsequent steps.
2. Add 20 μ l Proteinase K (20 mg/ml), vortex immediately to mix thoroughly. Incubate at RT for 10 min. To help digest, mix by vortex the tube during the incubation.
3. Add 200 μ l CB, vortex immediately to mix thoroughly. The solution should be clear (but the color is slightly blackish).
4. Optional : If there is a significant amount of residual RNA and it needs to be removed, 20 μ l of RNase A (10 mg/) solution can be added after completing step 3, shaken to mix, and left at room temperature for 5-10 minutes.
5. Add 200 μ l isopropyl alcohol invert to mix (a precipitate may form at time). Transfer the resulting solution and any possible precipitate into a adsorption column AC (the adsorption column is placed inside a collection tube).
6. Centrifuge for 30 sec, 12,000 rpm, discard the flow-through and place the spin column back in the same collection tube.
7. Add 500 μ l 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 μ l of Buffer WB to the spin column and centrifuge for 30 sec, 12,000 rpm .
9. Repeat step 8.
10. Discard the flow-through and place the spin column back in the same collection tube, centrifuge for an additional 2 min, 12,000 rpm.
11. Place the spin column into a clean 1.5 ml microcentrifuge tube. Add 50-100 μ l 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 $^{\circ}$ C to increase the elution effect) .
Note: The volume of elution buffer should not be less than 30 μ l, 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 $^{\circ}$ C, and for long-term storage, it can be kept at -20 $^{\circ}$ C