Cell/Tissue/blood DNA Extraction Kit

Code No.： NG416S 100 T
NG416M 200 T

Contents	storage	100 T	200 T
1×PBS	RT	40 ml	80 ml
Buffer HTL	RT	20 ml	40 ml
Buffer HB	RT	20 ml	40 ml
Buffer WB	RT	25 ml	50 ml
		Add absolute alcohol before use	
Buffer IR	RT	50 ml	100 ml
Buffer EB	RT	15 ml	20 ml
Protease K	-20 ℃	2 ml	4 ml
Spin Columns AC	RT	100	200
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 ℃ 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 HTL, HB 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.

Operation Procedure:

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

1. Sample preparation:

   A: Tissue culture cells

   Collect about 10^5-10^6 suspended cells into a 1.5 ml centrifuge tube; for adherent cells, they should be digested with trypsin and then pipetted up and down to collect. Centrifuge at 12,000 rpm for 10 seconds to pellet the cells. Discard the supernatant, stay about 10-20 µl of residual liquid. Add 200 µl of 1×PBS to resuspend and wash the cell, centrifuge at 12,000 rpm for 10 seconds to pellet the cells. Completely aspirate the supernatant, and resuspend the cell pellet in 180 µl HTL. Follow step 2.

B: Animal tissue

Take 20-50 mg of fresh or thawed tissue ground into a fine powder in liquid nitrogen, transfer it into a 1.5 ml centrifuge containing 180 µl HTL, and mix well by pipetting with a wide-bore tip. Follow step 2.

C: Animal tissue (mouse tail)

Cut 0.2-0.5 cm of the rat tail tip (i.e., 20-50 mg) into small pieces, the tissue into a fine powder in liquid nitrogen, transfer it into a 1.5 ml centrifuge tube containing 180 µl buffer HTL, and well by pipetting up and down with a wide-bore pipette tip. Follow step 2.

D: Blood

Take 200 µl of fresh, frozen, or anticoagulated blood and place it into a 1.5 ml centrifuge tube containing 180 µl buffer HTL (For large volumes of blood, select code No.405S or 406S). If the initial volume of whole blood is less than 200 µl, make up to 200 µl with 1×PBS. If the initial volume is between 200 µl and 300 µl, 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 µl, which can be made up to 200 µl with 1×PBS before proceeding to the subsequent steps.

2. Add 20 µl Proteinase K (20 mg/ml) and vortex immediately to mix thoroughly.
3. Incubate at 56 ℃ for 10 min (or until the tissue is completely digested). To help digest, mix by vortex the tube during the incubation.
4. Optional：If there is a significant amount of residual RNA and it needs to be removed, 20 µl 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 µl HB (20 mg/ml) and vortex immediately to mix thoroughly, incubate at 70 °C for 10 min.
6. Cool to room temperature, then add 200 µl 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).
7. Centrifuge for 30 sec, 12,000 rpm, discard the flow-through and place the spin column back in the same collection tube.
8. 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.
9. 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.
10. Repeat step 9.
11. Discard the flow-through and place the spin column back in the same collection tube, centrifuge for an additional 2 min, 12,000 rpm.
12. Place the spin column into a clean 1.5 ml microcentrifuge tube. Add 30-80 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.
13. DNA can be stored at 2-8 ℃, and for long-term storage, it can be kept at -20 ℃