Cell/Tissue DNA Extraction Kit (solution-type)

Code No.： NG402S 100 T
NG402M 200 T

Contents	storage	100 T	200 T
Buffer CN	RT	60 ml	120 ml
Buffer PD	RT	20 ml	40 ml
RNase A(10 mg/ml)	-20 ℃	200 µl	400 µl
Buffer EB	RT	15 ml	30 ml
filter column	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：

This kit is used for the rapid extraction of genomic DNA from animal cells/tissues. After sample grinding or homogenization, nuclear lysis buffer CN is added. First cells are lysed to release genomic DNA under the synergistic action of strong detergents. Then, RNase A is added to remove RNA, followed by the selective precipitation and of proteins using protein precipitation solution. Finally, the purified genomic DNA is precipitated by isopropanol and redissolved.

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 CN 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:

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 1X PBS to resuspend and wash the cell, centrifuge at 12,000 rpm for 10 seconds to pellet the cells. For cell lines that are not well lysed by the buffer CN (e.g., PC12 cells), a few freeze-thaw cycles be performed before proceeding to the next step: freeze in liquid nitrogen, then thaw in a 95 ℃ water bath, repeating this process 4 times. Completely aspirate the supernatant, and resuspend the cell pellet in 600 µl buffer CN. Follow step 2.

B: Animal and plant tissues

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 600 µl buffer CN, and mix well by pipetting with a wide-bore tip. Incubate at 65 ℃ for 15-30 min. 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 120 µl 0.5 M EDTA（pH8.0）and 500 µl buffer CN, and well by pipetting up and down with a wide-bore pipette tip. Add 20 µl Proteinase K (20 mg/ml) and vortex immediately to mix thoroughly. Incubate at 55 ℃ overnight (or until the tissue is completely digested). Follow step 2.

2. Add 1.8 µl RNase A (10 mg/ml), mix, 37 ℃, 15-30 min, then cool to room temperature.
3. Add 200 µl buffer PD and vortex immediately to mix thoroughly (a precipitate may form at time), ice bath 5 min.

   Due to the small volume and weight of the sample, the shear force generated by mixing with a vortex mixer will not shear and break the genomic DNA. Mixing by hand, do not shake vigorously up and down; only shake with moderate force, otherwise the genomic DNA will be sheared. However, the force should not be too light to thorough mixing and to break up the viscous lysate; otherwise, the DNA will not separate from the protein precipitate and will precipitate along with the protein during centrifugation, resulting in DNA loss reduced yield. In addition, insufficient mixing may also lead to incomplete protein precipitation, causing the final product to be contaminated with a significant amount of protein. Therefore, the use of a mixer is recommended.
4. centrifuge for 5 min, 13,000 rpm. Protein precipitates should be visible at the bottom of the tube, and some protein precipitates may also be seen floating on the surface of the liquid.
5. Pour the supernatant into the filter column, centrifuge at 12,000 rpm for 20 sec, and collect the filtrate into a 1.5 ml centrifuge tube.
6. Add an equal volume of room-temperature isopropanol (approx. 600 µl), invert 30 times to mix or until cotton-like (stringy) white DNA precipitate appears.

   Note: During inversion and mixing, the cotton-like (filamentous) DNA sometimes adheres to the cap or the tube rim and does not come down even with, which may lead the operator to miss the pellet and mistakenly assume that no DNA was obtained. The solution is to skip step 7, centrifuge directly at 12,000 rpm for 1 min, discard the supernatant, and then proceed to step 8.
7. Place the centrifuge tube vertically, allow the white DNA pellet to settle naturally to the bottom of the tube, then aspirate as much of the supernatant as possible taking care not to disturb the pellet.

   If the cotton-like (filamentous) DNA precipitate is attached to air bubbles, it will float on the liquid surface instead of settling down; be careful to the precipitate when pipetting the supernatant.
8. Add 1 ml 70% ethanol, invert and wash the DNA precipitate, centrifuge at 12,000 rpm for 1 min, a white DNA precipitate pellet can be seen at bottom of the tube, discard the supernatant.
9. Repeat step 8. Invert the tube and tap it gently on absorbent paper to drain the residual ethanol; you can also use a pipette tip to carefully aspirate the residual ethanol around precipitate at the bottom of the tube and on the tube walls, then air-dry the precipitate for a few minutes.

   Be careful not to over-dry it, otherwise the DNA will be extremely difficult to dissolve; nor should too much ethanol remain, otherwise the ethanol may inhibit downstream such as enzyme digestion.
10. Add 100 µl EB to rehydrate and dissolve the DNA pellet, flick the tube wall to mix, it can be incubated at 65 ℃ for 30-60 min (do not exceed one hour), flicking the tube wall occasionally during this time to help redissolve the DNA. Alternatively, it can be left overnight at room temperature or 4 ℃ to rehydrate the DNA.
11. DNA can be stored at 2-8 ℃, and for long-term storage, it can be kept at -20 ℃