Blood DNA Extraction mini Kit (solution-type)

Code No.： NG403S 100 T
NG403M 200 T

Contents	storage	100 T	200 T
10×Buffer RCL	RT	20 ml	40 ml
Buffer CL	RT	40 ml	80 ml
Buffer PD	RT	15 ml	30 ml
Buffer EB	RT	10 ml	20 ml

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 blood. First, the red blood cell lysis solution RCL lyses and removes red blood cells that do not contain DNA, and the cell nucleus lysis solution CL lyses white blood to release genomic DNA; then, the protein precipitation solution PD selectively precipitates and removes proteins; finally, the purified genomic DNA is precipitated by isopropanol and redissolved in the DNA solution EB.

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 CL 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. Add 900 µl ddH_2O and 100 µl 10×red blood cell lysis buffer RCL (diluted 1×) into a 1.5 ml centrifuge tube.
2. Invert the anticoagulated whole blood (returned to room temperature before use) to mix, then pipet 300 µl and add it the centrifuge tube containing the RCL from the previous step. Invert 6-8 times and gently flick the tube wall to ensure thorough mixing.
3. Leave at room temperature for 10 min (during which time, gently invert and flick the tube several times to mix and help lyse the red cells).
4. Centrifuge at 12,000 rpm for 20 sec, discard the red supernatant, and carefully aspirate as much supernatant as (be careful not to aspirate the cell pellet at the bottom of the tube), leaving the intact white cell pellet at the bottom of the tube and approximately 50 µl of supernatant.
   After centrifugation, a white pellet of white blood cells should be seen at the bottom of the tube, and there may also be some red cell debris mixed with white cell pellet. However, if a mostly red cell pellet is observed, it indicates that the red cell lysis was very insufficient, and steps 1-4 should be repeated to lyse the red cells.
5. Vortex until the white blood cell pellet is fully resuspended and dispersed.
   The resuspension and dispersion of white blood cells are crucial for the subsequent lysis step. If the white blood cells are not fully dispersed before adding the nuclear lysis, they will not be completely lysed, resulting in the formation of visible clumps.

6. Add 400 µl of buffer CL to the resuspended white blood cell, pipette up and down to lyse the leukocytes, or vortex vigorously for 10 sec to help lyse the leukocytes.
7. Optional step: generally not required: Add RNase A (10 mg/ml) to the lysate to a final concentration of 30 µg/ml, mix by inverting 25 times, incubate at 37 ℃ for 15 min to remove residual RNA, and then cool back to room temperature.
8. Add 150 µl buffer PD and vortex immediately to mix thoroughly (a precipitate may form at time).
9. Centrifuge for 5 min, 13,000 rpm. Protein precipitates should be visible at the bottom of the tube.
10. Pour the supernatant into a 1.5 ml centrifuge tube. When collecting the supernatant, be careful not to aspirate the bottom of the tube or the protein precipitates floating on the liquid surface. If the protein precipitates are accidentally into a new centrifuge tube, you can centrifuge again for 2 minutes and then collect the supernatant.
11. Add an equal volume of room-temperature isopropanol (approx. 500 µl), invert 30 times to mix or until cotton-like (stringy) white DNA precipitate appears.
12. Centrifuge at 12,000 rpm for 1 min, a white DNA precipitate pellet can be seen at bottom of the tube, discard the supernatant.
13. 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.
14. Repeat step 13. 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.
15. 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.
16. DNA can be stored at 2-8 ℃, and for long-term storage, it can be kept at -20 ℃.