bacterial DNA Extraction Kit (solution-type)

Code No.： NG409S 100 T
NG409M 200 T

Contents	storage	100 T	200 T
RNase A(10 mg/ml)	-20 ℃	250 μl	500 μl
Buffer CL	RT	60 ml	120 ml
Buffer PD	RT	20 ml	40 ml
Buffer EB	RT	15 ml	30 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 bacterial. First, the cell nucleus lysis solution CL lyses bacterial 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. Collect 1 mL of overnight cultured bacteria and add it to a 1.5 mL centrifuge tube.
2. Centrifuge at 9,000 rpm for 30 seconds to pellet the cells, discard the supernatant, and vortex or gently flick to resuspend the cell pellet. For Gram-positive bacteria, proceed to step 3. For Gram-negative bacteria, proceed directly to step 6.
3. Add 480 μl of 50 mM EDTA to completely resuspend the cells.
4. Add 120 μl lysozyme (20 mg/ml in 10 mM Tris-HCl, pH 8.0), mix well.
   For most Gram-positive bacteria such as: Bacillus subtilis, Micrococcus luteus, Arthrobacter luteus, Nocardia otitidiscaviarum, Rhodococcus rhodochrousand and Brevibacterium albidium, can be effectively lysed using lysozyme. However, for certain species of Staphylococcus, 60 μl of lysozyme (20 mg/ml) and 60 μl of lysostaphin (20 mg/ml) should be added to ensure effective lysis.
5. Incubate at 37 ℃ for 30-60 min, centrifuge at 12,000 rpm for 2 min, discard the supernatant, and vortex or gently flick to resuspend the cell pellet.
6. Add 300 μl buffer CL to the resuspended cell, pipette up and down to lyse the cell.
7. Incubate at 80 ℃ for 5 min, then cool back to room temperature.
8. Add 1.8 μl RNase A (10 mg/ml) to the lysate to a final concentration of 30 μg/ml, mix well, incubate at 37 ℃ for 15-60 min to remove residual RNA, and then cool back to room temperature.
9. Add 200 μl buffer PD and vortex immediately to mix thoroughly (a precipitate may form at time).

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, you must not shake it vigorously up and down, but only with moderate force, otherwise the genomic DNA will be sheared; however, the force cannot be too small, as it must ensure thorough mixing 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 DNA loss or 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 of a vortex mixer is recommended.

10. Centrifuge for 5 min, 13,000 rpm. Protein precipitates should be visible at the bottom of the tube.
11. Pour the supernatant into a new 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.
12. 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 that sometimes the cotton-like (filamentous) DNA sticks to the lid or the tube opening during inversion and mixing, and does not come down even inversion. This leads the operator to not see the pellet and mistakenly believe that no DNA was obtained. The solution is to skip step 13, directly centrifuge at 12000 rpm for 1 minute, discard the supernatant, and then proceed to step 15.
13. 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 possible, taking care not to disturb the pellet.
14. 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.
15. Repeat step 14. 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.
16. 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.
17. DNA can be stored at 2-8 ℃, and for long-term storage, it can be kept at -20 ℃.