bacterial DNA Extraction Kit

Code No.： NG408S 100 T
NG408M 200 T

Contents	storage	100 T	200 T
Buffer PHY	RT	10 ml	20 ml
Buffer PBS	RT	25 ml	50 ml
Buffer RB	RT	25 ml	50 ml
Buffer CB	RT	25 ml	50 ml
Buffer WB	RT	25 ml	50 ml
		Add absolute alcohol before use	
Buffer IR	RT	50 ml	100 ml
Protease K (Optional 20 mg/ml)	-20 ℃	2 ml	4 ml
Buffer EB	RT	15 ml	20 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 RB, 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.

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 ℃ 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 0.5-2 mL of culture medium (no more than 2×10^9 cells), centrifuge at 10000 rpm for 30 seconds, aspirate the supernatant as much as possible, and collect the bacterial cells. The initial processing volume can be adjusted based on bacterial, cell type, and expected yield. If the count of bacterial cells exceeds the maximum adsorption capacity, the yield will be severely reduced.
2. Add 200 µl PBS resuspension of precipitate, vortex immediately to mix thoroughly. Centrifuge for 30 sec, 10,000 rpm, discard the supernatant.
   Note: For Gram-positive bacteria that are difficult to lyse, step 2 can be skipped, and lysozyme can be added for cell wall. The specific method is as follows: add 180 µl of buffer (20 mM Tris-Hcl, pH 8.0; 2 mM Na_2-EDTA; 1.2% Triton X-100; add lysozyme to a final concentration of 20 mg/ml just before use (lysozyme must be by dissolving lysozyme powder in the buffer, otherwise it will be inactive)), and treat at 37 ℃ for more than 30 min.
3. Resuspend the cells thoroughly in 180 µl RB buffer by vortexing or pipetting.
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 20 µl Proteinase K (20 mg/ml), vortex immediately to mix thoroughly. Incubate at 65 ℃ for 10 min. To help digest, mix by vortex the tube during the incubation.
6. Add 200 µl CB, vortex immediately to mix thoroughly.
7. Add 100 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).
8. Centrifuge for 30 sec, 12,000 rpm, discard the flow-through and place the spin column back in the same collection tube.
9. 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.
10. 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.
11. Repeat step 10.
12. Discard the flow-through and place the spin column back in the same collection tube, centrifuge for an additional 2 min, 12,000 rpm.
13. 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）. DNA stored at -20 ℃.
    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.