High Purity Plasmid Maxi Extraction Kit (Fast Type)

Code No.: NG221 10 T

Contents	storage	10 T
RNaseA（100 mg/ml） -	-20 ℃	500 ul
Buffer P1	RT	100 ml
buffer P2	RT	100 ml
buffer P4	RT	100 ml
Buffer PHY	RT	30 ml
Buffer WB	RT	50 ml Add absolute alcohol before
Buffer EB	RT	20 ml
Spin Columns D8	RT	10
Collection tubes(50 ml)	RT	20
Injector(20 ml)	RT	10

Storage:

1. At first use, all the RNase A in the kit was added to buffer P1 and stored at 2-8 ℃，Time limit for 3-5 months. 5 months later，you can add RNase A again.
2. 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.
3. 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：

The modified SDS-alkaline lysis was used to lyse the cells, the new lysis buffer allows the adsorption of DNA onto silica membrane in the presence of high salt., and then the impurities and other bacterial components were removed by rinsing solution, finally, the purified plasmid DNA was eluted from the silica matrix membrane by low salt and high pH elution buffer.

Product Specifications：

1. The kit is quick and easy.
2. 0.2-1.5 mg of pure high-copy plasmid DNA could be rapidly extracted from 150-200 ml Escherichia coli.
3. The unique technology of kit can remove endotoxin effective and the transfection effect is excellent.

Operation Procedure:

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

1. Introduction on the use of buffer PHY: Silica gel membrane column placed long-term placement will react with the charge or dust in the air and affect its nucleic acid binding capacity. The ability of the silica gel column binding nucleic acid can be improved which pretreated with buffer PHY. Therefore, the output of plasmid can be improved. buffer PHY is a strong alkaline solution, if touched, please use a lot of water cleaning. Close the cap tightly after use to avoid contact with air. Store at room temperature. There may be precipitate formation during storage, please heat to 37 ℃ to make the precipitate completely disappear.

2. Usage: take a new Spin Column D8 in the Collection tube, add 2.5 ml of buffer PHY to the column, 8,000 rpm centrifuge for 3 min, Discard the flow-through and place the spin column back in the same collection tube, follow the next step.
3. Harvest 150-200 ml bacterial cells in a centrifuge tube by centrifugation at 8,000 rpm for 3 min at room temperature, then remove all traces of supernatant until all medium has been drained. For large amount of bacteria culture, collect the bacteria pellet into one centrifuge tube by splitting the bacteria culture into several centrifugation steps.
4. Resuspend pelleted bacterial cells in 10 ml Buffer P1 by Vortex oscillates to complete suspension. (Ensure that RNase A have been added to Buffer P1).
   Note: Cell clumps indicate incomplete lysis, which will result in lower yield and purity
5. Add 10 ml Buffer P2 and mix gently turned up and down 6-8 times to lyse fully. Placed at room temperature for 4-5 min.
   Note: Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. If not clear, probably due to incomplete lysis, please reduce the cells.
6. Add 10 ml buffer P4, immediately turn up and down gently 6-8 times, when fully mixed, the solution should become cloudy. Centrifuge at 8,000 rpm for 10-15 min and carefully remove the supernatant.
   Note: To avoid localized precipitation, mix the solution quickly, immediately after addition of Buffer P4.
7. All the supernatant after centrifugation in step 4 turn in injector. Please avoid pouring in a large amount of precipitate to prevent clogging the syringe; push the plunger slowly to filter, and collect the filtrate in a clean new 50 ml centrifuge tube.
8. Add isopropanol with 0.3 × the volume of the above filtrate, invert and mix 6-8 times, let stand for 2 min.
9. Transfer the solution to the Spin Column D8 (keep the spin column in a 50 ml collection tube) centrifuge for 2 min, 8,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
   Note: Because the maximum volume of the Spin Column D8 is 15 ml, so the solution needs to be split into two or more times loading in the Spin Column.
10. Add 10 ml of Buffer WB to the Spin Column and centrifuge for 2 min, 8,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
11. Add 10 ml of Buffer WB to the spin column and centrifuge for 2 min, 8,000 rpm again. Discard the flow-through and place the Spin Column back in the same collection tube, centrifuge for an additional 5 min, 8,000 rpm.
12. Place the Spin Column into a clean 50 ml centrifuge tube.
13. Add 1-2 ml of Buffer EB or water to the center of the membrane, let the column stand for 3 min, and then centrifuge for 2 min, 8,000 rpm（The elution buffer can be preheated at 65-70 ℃ to increase the elution effect）. To increase the plasmid recovery efficiency, the obtained solution can be re-added to the adsorption column and step 11 repeated. Then stored -20 ℃.
    Note: The volume of elution buffer should not be less than 1 ml, otherwise it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting.