Rapid Endotoxin-Free Plasmid Midiprep Kit

Code No.： NG218S 50 T
NG218M 200T 50 T

Contents	storage	50 T	200 T
RNaseA（100mg/ml） -	-20 ℃	300 ul	1200 ul
Buffer P1	RT	30 ml	120 ml
buffer P2	RT	30 ml	120 ml
buffer P4	RT	30 ml	120 ml
Buffer PHY	RT	10 ml	40 ml
Buffer WB	RT	15 ml Add absolute alcohol before use	60 ml
Buffer EB	RT	10 ml	40 ml
Spin Columns AC	RT	50	200
Collection tubes(2 ml)	RT	100	400
Columns FC(2 ml)	RT	50	200

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. The unique technology of kit can remove endotoxin effective and the transfection effect is excellent.

NOTE:

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 AC in the Collection tube, add 200 µl of buffer PHY to the column, 12,000 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 5-15 ml bacterial cells in a centrifuge tube by centrifugation at 12,000 rpm for 1 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 500 µl 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 500 µl 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 500 µl buffer P4, immediately turn up and down gently 6-8 times, when fully mixed, the solution should become cloudy. Centrifuge at 12,000 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 Columns FC (keep the spin column in a 2 ml collection tube), and collect the filtrate in a clean new 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 AC (keep the spin column in a 2 ml collection tube) centrifuge for 1 min, 12,000 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 AC is 700 µl, so the solution needs to be split into two or more times loading in the Spin Column.
10. Add 600 µl of Buffer WB to the Spin Column and centrifuge for 1 min, 12,000 rpm. Discard the flow-through and place the Spin Column back in the same collection tube.
11. Add 600 µl, of Buffer WB to the spin column and centrifuge for 1 min, 12,000 rpm again. Discard the flow-through and place the Spin Column back in the same collection tube, centrifuge for an additional 2 min, 12,000 rpm.
12. Place the Spin Column into a clean centrifuge tube.
13. Add 100-200 µl of Buffer EB or water to the center of the membrane, let the column stand for 3 min, and then centrifuge for 2 min, 12,000 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 13repeated. Then stored -20 ℃.
    Note: The volume of elution buffer should not be less than 100 µl, otherwise it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting.