Endotoxin-Free Plasmid Midi Kit

Code No.: NG213 50 T

Suitable for 5-15 ml medium-scale plasmid preparations (midipreps)

Contents	storage	50 T
RNaseA（10 mg/ml） -	-20 ℃	5 mg
Buffer P1	4 ℃	30 ml
buffer P2	RT	30 ml
buffer N3	RT	15 ml
buffer N4	RT	70 ml
buffer PE	-20 ℃	30 ml
Buffer WB	RT	20 ml Add absolute alcohol before use
Spin Columns AC	RT	50
Buffer EB	RT	15 ml
Collection tubes	RT	50

Storage Instructions:

1. Upon first use, add the entire RNase A provided with the kit to Solution P1 (final concentration 100 μg/ml) and store at 2-8 ℃. If RNase A in Solution P1 becomes inactive, trace amounts of RNA may remain in the extract plasmid; simply replenish RNase A in Solution P1.

2. When ambient temperatures are low, SDS in Solution P2 may precipitate, causing turbidity or sedimentation. Heat the solution in a 37 ℃ water bath for a few minutes to restore clarity; do not shake vigorously to avoid excessive foaming.

3. To prevent evaporation, oxidation, and pH chang by prolonged exposure to air, promptly recap all solutions after use.

Product Introduction:

This kit employs an improved SDS-alkaline lysis method to lyse cells. A special endotoxin removal agent selectively binds and centrifugally removes endotoxins. Subsequently, the silica-based membrane within the centrifugal adsorption column selectively binds plasmid the solution under high-salt, low-pH conditions. Impurities and other bacterial components are removed by Protein Removal Solution and Wash Solution. Finally, pure plasmid is eluted from the silica-based membrane using a low-salt, high-pH elution buffer.

Product Features:

1. The difference of silica-based membrane in adsorption capacity is extremely small, and the reproducibility is good.

2. The amount of plasmid was related to bacterial culture concentration and plasmid copy number. In general, 15-70 μg plasmid can be isolated from 5-15 ml high-copy plasmids which inoculated on LB with suitable antibiotics and cultured for 14-16 hours. If the plasmids are low copy or larger than 10kb, the amount of bacteria should be increased appropriately, and the amount of P1, P2 and N3 should be increased proportionally.

3. Extremely low endotoxin content (<0.1 EU/μg DNA), excellent cell transfection efficiency.

Operating Steps:

Before first use, please add the specified amount of ethanol to the Wash Buffer WB and mix thoroughly, mark it. Add the entire volume of RNase A to Solution P1 and mix thoroughly, mark it; store at 2-8 ℃ after each use.

1. Take 5～15 ml of overnight culture, centrifuge at 9,000 rpm for 2 min, decant the sus completely as possible, and collect the bacterial pellet.
2. Resuspend the cell pellet in 500 μl of buffer P1, and vortex until completely suspended. Any unmixed cell clumps will lysis, resulting in low yield and purity.
3. Add 500 μl of buffer P2, gently invert 6-8 times to fully lyse the cells, and it stand at room temperature for 4 minutes. Mix gently, do not shake vigorously to avoid shearing the genomic DNA! The total time should not exceed 5 minutes to prevent plasmid. At this point, the cell suspension should become clear and viscous. If there are few cells, you can proceed to the next step as soon as it becomes clear and viscous; does not have to be exactly 5 minutes.
4. Add 250 μl of buffer N3, and immediately invert gently 6-8 times. A white flocculent precipitate will when fully mixed. Centrifuge at 12,000 rpm for 10 minutes, and carefully transfer the supernatant to a new tube, avoiding the aspiration of floating white precipitates.
5. Transfer 0.95 ml of supernatant to a 2.0 ml centrifuge tube, add an equal volume of N4, and mix by verting.
6. Transfer the solution obtained in step 5 step to the AC adsorption column (place the adsorption column into a collection tube) in two portions (no more than 750 μl each). Centrifuge 12,000 x g for 1 minute, and discard the waste liquid in the collection tube. Repeat until all the mixed solution has passed through the adsorption column.
7. Add 500ul of PE. Centrifuge at 12000 rpm for 30 s.
8. Add 600 μl of Wash Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12,000 rpm for 30 seconds, and discard the waste liquid. Add another 600 μl of Wash Buffer WB and repeat the wash once.
9. Place the adsorption column AC back into an empty collection tube, centrifuge at 13,000 rpm for 2 minutes to remove as wash buffer as possible, to avoid residual ethanol in the wash buffer from inhibiting downstream reactions.
10. Remove the adsorption column AC and place it into a clean centrifuge tube. Add 80-100 μl of elution buffer EB preheating the buffer EB in a 65-70 ℃ water bath yields better results) to the center of the adsorption membrane. Let it stand at room temperature for 2 minutes, then centrifuge at 12,000 rpm for 1 minute. If a larger amount of plasmid is required, the obtained solution can be re-added the adsorption column, let it stand at room temperature for 2 minutes, and centrifuge for 1 minute.

The larger the elution volume, the higher the elution efficiency. If a higher plasmid concentration is required, the elution volume can be appropriately reduced, but the minimum should not be less than 50 μl. An excessively small volume will reduce the plasmid elution efficiency and decrease the plasmid yield.