Endotoxin-Free Plasmid Midi Kit

Code No.： NG203S 50 T

NG203 100 T

Contents	storage	50 T	100 T
RNaseA（10 mg/ml） -	-20 ℃	150 μl	300 μl
buffer PHY	RT	5 ml	10 ml
Buffer P1	4 ℃	15 ml	30 ml
buffer P2	RT	15 ml	30 ml
buffer N3	RT	15 ml	30 ml
buffer ED	-20 ℃	5 ml	10 ml
Buffer WB	RT	15 ml	25 ml
		Add absolute alcohol before use	
Spin Columns AC	RT	50	100
Buffer EB	RT	10 ml	15 ml
Collection tubes	RT	50	100

Storage Instructions:

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. 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. 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. 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. please check the box immediately to indicate that ethanol has been added to avoid repeated addition. Add the entire volume of RNase A to Solution P1 and mix thoroughly; store at 2-8 ℃ after each use.

1. Take 1.5～4.5 ml of overnight culture, centrifuge at 12,000 rpm for 30 seconds, decant the sus completely as possible, and collect the bacterial pellet. If more than 1.5 ml of culture is collected, centrifuge to discard the supernatant, add more cu to the same 1.5 ml tube, and repeat step 1 until sufficient bacterial pellet is collected.
2. Resuspend the cell pellet in 250 μl of buffer P1, and vortex until completely suspended. Any unmixed cell clumps will lysis, resulting in low yield and purity.
3. Add 250 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 13,000 rpm for 10 minutes, and carefully transfer the supernatant to a new tube, avoiding the aspiration of floating white precipitates. The mixture should be mixed immediately after adding solution N3 to prevent local precipitation of SDS.
5. Add 0.1 volume (10% of the supernatant volume, approximately 80 μl) of buffer ED to the supernatant in the previous step, mix by inverting, and place in an ice bath or embed in crushed ice (or in a freezer) for 5 minutes until the turbidity clears (or still slightly cloudy), mixing occasionally in between.
After adding the buffer ED to the supernatant, the supernatant will become turbid, but it should return to clear (or slightly turbid) after an ice.
6. Leave at room temperature for 3-5 minutes. Once the temperature returns to room temperature, the solution will quickly become turbid; invert to mix If the indoor temperature is low or you wish to speed up the process, you can use a 37-42 ℃ water bath, which will make it turn turb quickly; invert to mix.
7. Centrifuge at 14,000 x g for 10 minutes at room temperature to separate the phases. The upper aqueous phase contains, and the lower blue oily phase contains endotoxins and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (be careful not to aspirate the blue oily, which contains endotoxins and other impurities), and discard the oily layer.
(Optional step) Pre-treatment of the adsorption column with buffer PHY.
8. Add 0.5 volumes of isopropanol (approx. 370 μl) to the upper aqueous phase, mix thoroughly by verting, and transfer to the AC adsorption column (place the adsorption column into a collection tube) in two portions (no more than 700 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.
9. 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.
10. 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.

11. Remove the adsorption column AC and place it into a clean centrifuge tube. Add 50-100 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 30 µl. An excessively small volume will reduce the plasmid elution efficiency and decrease the plasmid yield.

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 prm centrifuge for 1 min, Discard the flow-through and place the spin column back in the same collection tube, follow the next step.