EndoFree Maxi Plasmid Kit

Cat #: orb1566789 (manual)

Size: 1 test / 10 tests

Product Composition

Reagent name	1 test	10 tests
Buffer A1	12 ml	125 ml
Buffer B1	12 mL	125 ml
Buffer C1	5 mL	50 ml
Buffer D1	25 mL	250 ml
Buffer PW	5 mL	50 ml
Buffer EB	3 mL	30 ml
RNase A (10 mg/ml)	0.125 mL	1.25 ml
Adsorption column	1	10
Collection tube (50ml)	2	20
Manual	1 copy	1 copy

Storage conditions

1. This reagent kit is transported at room temperature and can be stored for 12 months under dry conditions at room temperature. For longer storage periods, it can be stored at 2-8 ℃.

2. When the temperature is low, some solutions may precipitate. Before use, heat them in a 37 ℃ water bath until the precipitate disappears.

3. Before the first use, add RNase A to Buffer A1, mix well, and store at 2-8 ℃ with a shelf life of six months.

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4. RNase A can be stably stored at room temperature for more than 6 months, and long-term storage requires storage at -20 ℃.

Product Introduction

This kit uses a unique silica gel membrane adsorption technology to efficiently and specifically bind plasmid DNA. Simultaneously using endotoxin removal solution D1 can effectively remove impurities such as endotoxins and proteins; The entire extraction process only takes 1 hour, which is convenient and fast. The plasmid DNA extracted using this kit can be used for various conventional operations, including enzyme digestion, PCR, sequencing, ligation, transformation, and transfection of various cells.

Recommended dosage for each use of bacterial solution: The recommended dosage for high copy plasmids is 100 ml, and the yield is generally around 500-1500 μ g; The recommended dosage for low copy plasmids is 200 ml, and the yield is generally around 50-300 μg.

Operation Procedure

Before use, please add anhydrous ethanol to Buffer PW. Please refer to the label on the bottle for the added volume.

1. Inoculate the target strain in a medium containing appropriate antibiotics and shake at 37 ℃ for 12-16 hours.

2. Take 100-200 ml (choose the appropriate amount according to the concentration of the cultured bacterial cells, and recommend using 200ml for low copy) of the bacterial solution cultured overnight, add it to a centrifuge tube, centrifuge at 5000 × g for 10 minutes at room temperature, and try to remove the supernatant as much as possible (if there is a large amount of bacterial solution, multiple centrifugation can be used to collect the bacterial sediment in one centrifuge tube, and the amount of bacterial solution should be sufficient to fully lyse, as too much bacterial solution can lead to insufficient lysis and reduce the efficiency of plasmid extraction).

3. Add 10ml Buffer A1 to the centrifuge tube containing bacterial sediment (please check if RNase A has been added first), and use a pipette or vortex shaker to thoroughly suspend the bacterial sediment.

   Note: It is necessary to thoroughly suspend the bacterial cells, otherwise it will affect lysis and result in lower yield and purity. For low copy plasmids, increase the amount of bacterial cells while proportionally increasing the amount of Buffer A1, B1, and C1.

4. Add 10ml Buffer B1 to the centrifuge tube, gently invert the tube 6-8 times immediately to fully lyse the bacterial cells, and let it stand at room temperature for 5 minutes.

   Note: Mix gently and do not shake vigorously to avoid disrupting genomic DNA. At this point, the bacterial solution should become clear and viscous to prevent damage to the plasmid. If the bacterial liquid does not become clear, it may be due to excessive bacterial cells and incomplete lysis, and the bacterial volume should be reduced.

5. Add 5 ml of Buffer C1, gently flip it up and down 6-8 times, mix thoroughly, and a white flocculent precipitate will appear. Then let it stand at room temperature for about 10 minutes, transfer the centrifuge tube to a high-speed centrifuge at 10000 × g for 10 minutes, centrifuge the white precipitate to the bottom of the tube (the centrifugation time can be increased appropriately), and collect the supernatant in a clean 50 ml tube (self-prepared).

Note: After adding solution C1, it should be mixed immediately to avoid local precipitation. If there is still a white precipitate in the solution after centrifugation, it can be centrifuged again. If there are too many bacterial cells (>100 ml), it is recommended to extend the centrifugation time to 20-30 minutes.

6. Transfer the supernatant to a new 50ml centrifuge tube (if there is still a white precipitate in the supernatant, it can be centrifuged again). Please divide the supernatant into 2 tubes and add 0.9-1.0 times the volume of Buffer D1 and an equal volume of anhydrous ethanol (i.e. 9-10ml of Buffer D1 and 10ml of anhydrous ethanol are added to every 10ml of supernatant). Shake vigorously by hand 5 times to mix well. The solution should be immediately centrifuged through a DNA column.

7. Immediately transfer 20ml of the above mixture to the adsorption column (place the adsorption column into a 50ml collection tube), centrifuge at room temperature of 5000 × g for 2 minutes, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.

   Note: The maximum effective volume of the adsorption column is 20ml. If there is a large amount of cracking solution, it can be added multiple times until all of it flows through the adsorption column.

8. Add 10 ml of Buffer PW to the adsorption column (please check if anhydrous ethanol has been added first), centrifuge at room temperature of 5000 × g for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.

9. Repeat step 8 once.

10. Add 5 ml of anhydrous ethanol to the adsorption column, centrifuge at 5000 × g for 1 minute at room temperature, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.

11. Place the centrifuge tube back into the high-speed centrifuge, centrifuge at room temperature of 5000 × g for 10 minutes, then open the adsorption column cover and let it stand at room temperature for a few minutes to thoroughly dry the residual rinse solution in the adsorption material.

    Note: Residual ethanol can affect subsequent enzymatic reactions (such as digestion, PCR, etc.) experiments.

12. Place the adsorption column in a clean 50 ml collection tube, suspend 0.5-2 ml of elution buffer EB in the middle of the adsorption membrane, let it stand at room temperature for 5 minutes, then centrifuge at room temperature>5000 × g for 5 minutes. Transfer all the elution solution in the 50 ml centrifuge tube into a clean 1.5 ml centrifuge tube and store at -20 ℃.

    Note: To increase the efficiency of plasmid recovery, the obtained solution can be re added to the adsorption column and repeat step 12. The pH value of the eluent has a significant impact on the elution efficiency. If ddH_2O is used as the eluent, its pH value should be maintained within the range of 7.5-8.0, pH values below 7.0 will reduce elution efficiency. The amount of elution buffer used is mainly determined by the copy number of the plasmid and the concentration required for the experiment. The volume of elution buffer should not be less than 0.5 ml, as a small volume can affect the recovery efficiency. DNA products should be stored at -20 ℃ to prevent DNA degradation.

Precautions (Please be sure to read these cautions before using this kit.)

1. Before use, add RNase A to solution A1 (add all RNase A provided in the kit), mix well, and store at 2-8 ℃.

2. Before use, check whether there is crystallization or precipitation in solutions B1 and D1. If there is crystallization or precipitation, heat them in a 37 ℃ water bath for a few minutes to restore clarity.

3. Be careful not to directly contact solutions B1 and D1, and immediately cover the lid after use.

4. The amount of extracted plasmids is related to factors such as bacterial culture concentration and plasmid copy number. If the extracted plasmid is a low copy plasmid or a large plasmid larger than 10 kb, the amount of bacterial cells used should be increased, and the amounts of solutions A1, B1, and C1 should be proportionally increased; It is recommended to preheat the elution buffer EB in a water bath at 65-70 ℃. The adsorption and elution time can be appropriately extended to improve the extraction efficiency.

Detection of plasmid DNA concentration and purity

The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. An OD260 value of 1 is equivalent to approximately 50 μ g/ml of double stranded DNA. The purified plasmid DNA OD260/OD280 is usually around 1.8-2.0, which can be directly applied in experiments that require high DNA purity, such as cell transfection and even animal in vivo experiments.

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