Version: V01/2022

FinePure EndoFree Plasmid Maxi Plus Kit

(Column)

Catalog: D919

Kit Contents

Contents	D919 (10 preps)
Buffer BL	30 ml
Buffer P1	120 ml
Buffer P2	120 ml
Buffer P6	120 ml
Buffer ETR	60 ml
Buffer DW2	44 ml
Buffer EB	30 ml
RNase A (10 mg/ml)	1.2 ml
Spin Columns PM8	10 pcs
FinePure Maxi Filter Syringe	10 pcs
50 ml Collection Tubes	10 pcs

Storage

EndoFree Plasmid Maxi Plus Kit can be stored at room temperature (15~25) °C under dry conditions for up to 12 months. Add RNase A to Buffer P1 before first use, mix well and store at 2~8 °C. The mixture can be stored stably for more than 6 months.

This Product Is Special for Scientific Research

Introduction

This kit is suitable for extracting 100~300 ml of overnight cultured bacteria, adopting a unique silica membrane adsorption technique to efficiently and specifically bind plasmid DNA. By combining the special Endotoxin Removal Buffer (ETR) and FinePure Maxi Filter Syringe, endotoxin, proteins and other impurities can be effectively removed. The whole extraction process takes only 1 hour, ensuring the convenient and quick operation. The plasmid DNA extracted by this kit can be applied to various routine operations, including enzymatic digestion, PCR, sequencing, ligation, transformation as well as the transfection for various cell types.

Important Notes

Please read this note before using this kit.

1. RNase A should be added into Buffer P1 before use (add all the RNase A provided in the kit). Mix well and store at 2~8 °C.
2. Please add the anhydrous ethanol into Buffer DW2 before use, and refer to the label on the bottle for the ethanol volume.
3. Check if there's crystal or precipitate formed in Buffer BL, Buffer P2 or Buffer P6 before use. If crystal or precipitate are present, warm the solution in a 37 °C water bath for a few minutes until the buffers turn clean.
4. Be careful not to touch Buffer P2 and P6 directly, and tighten the cap immediately after use.
5. When using the FinePure Maxi Filter Syringe, carefully pull the plunger out of the filter tube to avoid loosening the filter membrane due to pressure.
6. The amount of the plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. If the plasmid is low-copy or is with the size larger than 10 kb, increase the bacterial culture amount, and the amount of Buffer P1, P2 and P6 should be increased in proportion. It is recommended to preheat the Buffer EB in a 65~70 °C water bath before use. The adsorption and elution time can be appropriately extended to improve the extraction efficiency.
7. Prior to each experiment, please balance Spin Column PM8 with Buffer BL to maximize the activation of the silicon matrix membrane and increase the yield.
8. The column balanced with Buffer BL should be used immediately, otherwise the effect will be affected.

Protocol

1. **Column equilibration:** Add 2.5 ml Buffer BL to the Spin Column PM8 **(place the Spin Column PM8 in a 50 ml Collection Tube)**. Centrifuge at 10,000 rpm/10,610 g for 1 min. Dispose the waste liquid in the collection tube, and put the spin column back in the collection tube **(the column treated with Buffer BL should be used immediately)**.
2. Transfer 100 ml overnight cultured bacteria media into a centrifuge tube (choose the proper amount according to the concentration of the cultured cells. For low-copy plasmids, 200 ml bacteria culture is recommended)**.** Collect the bacteria by centrifuging at 10,000 rpm/10,610 g for 5 min at room temperature. Dispose the supernatant as much as possible. Remove the supernatant as complete as possible. To ensure that the supernatant is completely removed, use a clean absorbent paper to remove the water droplets on the bottle wall.
   Note: For large amount of bacteria culture, collect the bacteria pellet into one centrifuge tube by splitting the bacteria culture into several centrifugation steps. Choose the amount of bacteria that can be fully lysed, because excessive bacteria will lead to insufficient lysis and reduce the extraction efficiency of the plasmid.
3. Add 10 ml of Buffer P1 **(Please check if RNase A has been added)** to the centrifuge tube with bacteria pellet. Completely resuspend the bacteria cell pellet by pipetting or vortex.
   Note: Please ensure the complete suspension of the bacteria pellet. If the bacteria pellet is not thoroughly mix, the lysis efficiency will be affected, resulting in low extraction amount and low purity. For low-copy plasmids, increase the bacterial culture amount, and the amount of Buffer P1, P2 and P6 should be increased in proportion.
4. Add 10 ml of Buffer P2 to the centrifuge tube, and gently invert the tube 8~10 times to fully lyse the cells. Incubate at room temperature for 5 min.
   Note: Mix gently, do not vigorously oscillate, as this will cause contamination of genomic DNA. After this step, the bacteria suspension should become clean and viscous, if not, it might be due to excessive bacteria cells and incomplete lysis, which can be avoided by reducing the bacteria amount.
5. Add 10 ml of Buffer P6 to the lysate from step 4, and gently invert the tube 6~8 times to fully lyse the cells till white dispersion flocculent precipitate appears. Incubate at room temperature for 10 min. Centrifuge at 10,000 rpm/10,610 g for 10 min until the precipitates are fully collected to the bottom of the tube (the centrifuge time can be extended accordingly). Carefully pour all the supernatant into FinePure Maxi Filter Syringe (please avoid pouring the precipitant in for they will block the filter). Gently push the plunger and filter the cell lysate into a clean 50

ml tube (self-prepared).

Note: Mix the lysate immediately after adding Buffer P6 to avoid local precipitation. The filtration will not be affected if the lysate poured into Spin Columns PM8 contain the white precipitate. If large quantity of bacteria is applied (> 100 ml), it is recommended to extend the centrifugation time to 20~30 min.

6. Add 5 ml of Buffer ETR to the filtrate and mix well by inversion. Centrifuge at 10,000 rpm/10,610 g for 5 min, carefully pipette the the upper layer into a new 50 ml centrifuge tube.

   Note: the centrifugation temperature should be above 20 °C, if not, the stratification will be affected. If the stratification cannot be effectively finished, it is recommended to increase the centrifugation temperature and prolong the centrifugation time. When pipetting the upper layer, do not suck up the red oily layer, which contains impurities such as endotoxin.

7. Add isopropanol with 0.3 × volume of the supernatant from step 6 to the solution in a 50 ml centrifuge tube (excessive isopropanol might easily cause RNA contamination), Mix by inverting and transfer the solution to the Spin Column PM8 (keep the spin column in a 50 ml collection tube).

   Note: Please adjust isopropanol quantity according to the real supernatant volume. Because the maximum volume of the Spin Column PM8 is 15 ml, so the solution needs to be split into two-time or three-time loading in the Spin Column PM8.

8. Centrifuge at 10,000 rpm/10,610 g for 1 min at room temperature and dispose the waste liquid in the collection tube, then place the Spin Column PM8 back in the collection tube.

   Note: Please divide the solution obtained from step 7 to the spin column into two-time or three-time loading, and perform the operation according to the above conditions for each time.

9. Add 10 ml of Buffer DW2 (please check if absolute ethanol has been added) to Spin Column PM8. Centrifuge at 10,000 rpm/10,610 g for 1 min and dispose the waste liquid in the collection tube, then place the spin column back in the collection tube.
10. Repeat step 9 once.
11. Add 3 ml of absolute ethanol to the adsorption column, centrifuge at 10,000 rpm/10,610 g at room temperature for 1 min, discard the waste liquid.
12. Put the adsorption column back into the collection tube, and centrifuge at 10,000 rpm/10,610 g for 3 min. The purpose is to remove the residual rinsing liquid from the adsorption column. Use a pipette tip to remove the residual ethanol between the inner ring and the column wall, and open the lid to dry at room temperature for 3~5 min.

    Note: The residual ethanol will affect subsequent enzymatic reactions such as enzymatic digestion, PCR, *etc.*

13. Place the Spin Column PM8 in a clean 50 ml collection tube, and add 1~2 ml of Buffer EB to

the middle area of the adsorption membrane (Buffer EB preheated at 65~70 ℃ is recommended for the higher yield). Stay at room temperature for 5 min and then spin at 10,000 rpm/10,610 g for 5 min at room temperature. Transfer the eluate from the 50 ml collection tube to a clean 1.5 ml microcentrifuge tube and store at -20 °C.

Note: To improve the recovery rate of plasmid DNA, it is optional to repeat step 13. The pH value of the elution buffer significantly affects the elution efficiency, so if ddH_2O is used for elution, please make sure the pH value is within the range of 7.5-8.0. if the pH value is below 7.0, the elution efficiency will be decreased. The volume of the elution buffer depends on the plasmid copy number and the concentration of plasmids required for the experiment. It is recommended that the volume of the elution buffer is 2 ml. If a higher concentration of plasmids or low-copy plasmids are required, 1 ml can be used. If the volume is too small, the recovery efficiency will be affected. DNA products should be stored at -20 ℃ to avoid DNA degradation.

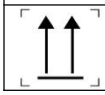
Optional Operation (for higher concentration of plasmids):

14. Add 710 µl of isopropanol and 210 µl of 5M NaCl (both the reagents self-prepare) to 500 µl of eluate in 1.5 ml microcentrifuge tube, mix well, stay at room temperature for 5 minutes, spin at 12,000 rpm/13,680 g for 10 minutes, and discard the supernatant carefully.
15. Add 500 µl of 70% ethanol to wash the precipitate, centrifuge at 12,000 rpm/13,680 g at room temperature for 5 min, and carefully discard the ethanol.
16. Repeat step 15.
17. Dry the precipitate in the air or 37 ℃ oven for 5~10 minutes, and dissolve the precipitate with an appropriate volume of Buffer EB.

Detection of Plasmid DNA Concentration and Purity

The purity and concentration of the extracted plasmid DNA can be detected by agarose gel electrophoresis and an ultraviolet spectrophotometer. An OD260 value of 1 corresponds to approximately 50 µg/ml of double stranded DNA. The OD260/OD280 value of the purified plasmid DNA is generally in the range of 1.7-1.9. The purified plasmid DNA can be directly applied to experiments with high purity requirements, such as cell transfection or in vivo experiments.

Symbols

Symbols	Meanings
	Catalogue number
	Batch code
	Date of manufacture
	Use-by date
	Manufacturer
	Do not use if package is damaged and consult instructions for use
	Up

Symbols	Meanings
	Consult instructions for use or consult electronic instructions for use
	Do not re-use
	Temperature limit
	Contains sufficient for <n> tests
	Keep dry
	Keep away from sunlight

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