FastPure EndoFree Plasmid Maxi Kit

Catalog #DC202

Version 8.1

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Introduction

FastPure EndoFree Plasmid Maxi Kit is specially designed for the purification of endotoxin-free plasmid DNA from 150 ml - 300 ml of overnight bacterial cultures. Based on an optimized SDS alkaline lysis technology, the endotoxin in crude extract selectively binds to a unique Endotoxin Removal Buffer and then removed by centrifugation, while the plasmid DNA selectively binds to the silicon membrane in the spin column under high-salt and low-pH condition. Then, the RNA, proteins, and other impurities are removed by the wash buffer. Finally, the plasmid DNA is eluted from silicon membrane in a low-salt concentration of buffer with high-pH. This kit needs no phenol, chloroform, or other reagents that are toxic, and needs no ethanol precipitation. Pure high-copy plasmid DNA of 0.2 mg - 1.5 mg can be quickly extracted with a yield rate of 80% - 90% and a endotoxin level of < 0.1 EU/μg plasmid DNA. The plasmid DNA obtained can be used for high-efficient cell transfection, and can also be used in a wide variety of molecular biology procedures, including restriction endonuclease digestion, PCR, in vitro transcription, transformation, and sequencing.

Components

Components	DC202-01
RNase A	750 μl
Buffer P1	75 ml
Buffer P2	75 ml
Buffer P4	75 ml
Endotoxin Removal Buffer	25 ml
Buffer PW	2 x 22 ml
Buffer TB	20 ml
FastPure DNA Maxi Columns(each in a 50 ml Collection Tube)	10 Columns

RNase A (10 mg / ml): for the removal of RNA.

Buffer P1: Bacteria suspension solution. Please add all the RNase A to Buffer P1 prior to use.

Buffer P2: Bacteria lysis buffer (contains SDS/NaOH).

Buffer P4: Neutralization buffer.

Endotoxin Removal Buffer: for the effective removal of endotoxin from the crude extract.

Buffer PW: Wash solution, please add proper volume of ethanol according to the instructions before the first use.

Buffer TB: for the elution of plasmid DNA.

Storage

RNase A should be stored at -30°C ~ -15°C and transported at -20°C ~ 0°C.
Endotoxin Removal Buffer can be stored at 2°C - 8°C for one month (at -30°C ~ -15°C for long-term), and transported at -20°C ~ 0°C.
Other components should be stored and transported at room temperature (15°C - 25°C).

Application

Applicable for large scale isolation of high-quality endotoxin-free plasmid DNA from 150 ml - 300 ml of overnight cultures.

Tips

◇ Before the first use, please add all the RNase A to Buffer P1, and store at 2°C - 8°C.

◇ Add proper volume of ethanol to dilute Buffer PW2 according to the instructions as indicated on the label, store at room temperature.

◇ The yield and quality of purified plasmid DNA may vary according to strain type, concentration of bacteria, plasmid copy number and plasmid stability. For low-copy plasmid or long-fragment plasmid (> 10 kb), to increase extraction efficiency, it is highly recommended to (1) increase the volume of starting culture; (2) increase the volume of Buffer P1, Buffer P2 and Buffer P4 in proportion; (3) pre-heat the Buffer TB to 55°C; and (4) extend the time for adsorption and elution.

◇ Buffer P2 and Buffer P4 may precipitate at low temperature. If precipitate is present, warm the solution in a 37°C water bath for 5 min to re-dissolve precipitation, and equilibrate to room temperature before use.

◇ Avoid direct contact with Buffer P2 and Buffer P4 with bare hands, wear gloves before performing and immediately close the lid after use.

◇ The concentration and purity of extracted plasmid DNA can be detected by agarose gel electrophoresis and an ultraviolet spectrophotometer. Electrophoresis may be a single band or two or more DNA bands, which is mainly caused by polymorphic plasmids (which have different degrees of super-helical conformation) in different migration positions, and the time of growing bacterial cultures, the intensity of the operation during extraction, etc. The degrees of super-helical conformation of plasmid is over 90% under normal operating by this kit.

◇ The exact molecular size of the plasmid DNA can only be determined after linearization. For intact plasmids in circular or super-helical state, the exact size cannot be de determined by electrophoresis, due to the uncertain migration on the gel.

Principle and Procedure

Re-suspend	Add 7.5 ml of Buffer P1
Lyse	Add 7.5 ml of Buffer P2
Neutralize	Add 7.5 ml of Buffer P4
Remove Endotoxin	Add 0.1 volume of Endotoxin Removal Buffer to supernatant
Bind	Add 0.5 volume of Isopropanol
Wash	Add 10 ml of Buffer PW
	Add 10 ml of Buffer PW
Elute	Add 1 ml - 2 ml of Buffer TB or endotoxin-free water

Additional Materials Required

Ethanol, Isopropanol, 50 ml high-speed centrifuge tubes.

Protocol

1. Harvest 150 ml - 200 ml (no more than 300 ml) of overnight cultured bacterial cells into a centrifuge tube, centrifuge at 12,000 x g (approximately 10,000 rpm) for 1 - 2 min, discard the supernatant.

▲ Collecting more than 50 ml of culture, please harvest cells in one tube by repeated centrifugation steps.

2. Add 7.5 ml of Buffer P1 (add RNase A before use), mix thoroughly by vortex or pipetting up and down.

▲ No cell clumps should be visible. Complete resuspension of cell pellet is vital to high yield and purity of plasmid DNA.

3. Add 7.5 ml of Buffer P2, and mix thoroughly by inverting the tube gently for 6 - 8 times, then incubate at room temperature for 4 - 5 min.

▲ Gently invert the tube (**DO NOT vortex**, because vortexing may lead to the shearing of genomic DNA) to get a clear lysate. The lysis should last for less than 5 min to prevent plasmid breaking. If the lysate is not clear, please reduce bacterial cell input properly.

4. Add 7.5 ml of Buffer P4, and mix immediately and thoroughly by inverting the tube gently for 6 - 8 times to neutralize Buffer P2 until a flocculent white precipitate forms, centrifuge at 12,000 x g for 10 - 15 min. Pipet carefully the supernatant into a new tube without disturbing the precipitate.

▲ Immediately invert the tube and mix thoroughly after adding Buffer P4 to avoid localized precipitation that may affect the neutralization.

5. Add 0.1 volume of Endotoxin Removal Buffer to the supernatant from **Step 4** (i.e., add 2.2 ml of Endotoxin Removal Buffer to 22 ml of supernatant), mix thoroughly by inverting the tube. Then place in ice bath or insert into crushed ice (or freezer) for 5 min until the solution becomes clear and transparent (or still slightly turbid) from the turbidity, occasionally mixing several times during this period.

▲ After Endotoxin Removal Buffer is added to the supernatant, the supernatant becomes turbid, but should be clear (or slightly turbid) after the ice bath.

6. Incubate at room temperature for 3 - 5 min. The solution quickly became turbid after the temperature returned to room temperature. Then mix thoroughly by inverting the tube upside down.

▲ If the room temperature is low or want to speed up, it can become turbid quickly in the water bath at 37°C - 42°C.

7. Centrifuge at 14,000 × g for 10 min at room temperature. The solution is now separated into two layers. Upper layer contains DNA and lower blue oily layer contains endotoxin and other impurities. Carefully collect the upper layer without disrupting the lower layer and transfer it to a new centrifuge tube.

▲ Avoid disrupting the lower blue oily layer which contains endotoxin and other impurities.

▲ If it cannot be effectively layered. The centrifugation temperature should be adjusted to 30°C and prolong the centrifugation time to 15 min.

8. Add 0.5 volume (approximately 10 ml) of isopropanol to the upper layer suspension from **Step 7**. Mix thoroughly by inverting the tube, and transfer the solution to the adsorption column by several times (The adsorption column is pre-assembled with collection tube). Centrifuge at 12,000 × g for 1 min and discard the supernatant from the collection tube. Please harvest to one tube by several centrifugation steps, until all the mixed solution passed through the adsorption column.

▲ The capacity of adsorption column is 15 ml. Be careful not to overfill the column. If the centrifuge uses an angular rotor, it is recommended that the volume of the column not exceed 12 ml.

9. Add 10 ml of Buffer PW (with ethanol added in) to the Filtration Column, centrifuge at 12,000 × g for 1 min, discard the filtrate and reuse the Collection Tube.

10. Repeat **Step 9**.

11. Use a tip to remove any ethanol that may remain between the inner pressure ring and the column wall. Place the column back into the same empty collection tube, and centrifuge at the highest speed (preferably higher than 12,000 × g) for 3 min to dry the residual ethanol on the adsorption film. Open the lid and let it dry for 3 - 5 min at room temperature.

▲ Ethanol residues can affect downstream enzymatic reactions such as digestion, enzyme ligation, PCR, etc. **DO NOT skip this step**.

12. Insert the Column into a new tube. Add 1 ml - 2 ml of Buffer TB (Pre-heating of Buffer TB in a 55°C water bath can increase yield) to the middle of the adsorption film. Incubate at room temperature for 3 min. Centrifuge at 12,000 × g for 3 min, elute plasmid.

▲ In order to increase the recovery efficiency of the plasmid, the obtained solution can be transferred into the adsorption column again, incubate at room temperature for 3 min, and centrifuged at 12,000 × g for 3 min. An additional elution step (optional) can increase the concentration by about 10%.

▲ Larger elution volume leads to higher elution efficiency. However, for higher plasmid concentration, the elution volume should be reduced appropriately. Be aware that lower volumes will reduce the efficiency of plasmid elution, but reduce the plasmid yield (minimum volume should not be less than 1 ml).

13. Discard the purification column. Use the purified plasmid DNA for downstream applications, or store at -20°C.

Troubleshooting

Problem	Possible Reason	Recommended Solutions
Low plasmid DNA yield	1. Low copy-number plasmid	The yield of plasmid varies depending on copy number. * Low copy-number plasmid: pBR322, SuperCos, pWE15, pACYC (derivatives), and pSC101 (derivatives) * High copy-number plasmid: pTZ, pUC, pBS, pGM-T. For low-copy plasmid, it is highly recommended to: (1) increase the volume of starting culture; (2) increase the volume of Buffer P1, Buffer P2 and Buffer P4 in proportion; (3) pre-heat the Buffer TB to 55°C; and (4) extend the time for adsorption and elution.
	2. Long-fragment plasmid (> 10 kb)	it is highly recommended to (1) increase the volume of starting culture; (2) increase the volume of Buffer P1, Buffer P2 and Buffer P4 in proportion; (3) pre-heat the Buffer TB to 55°C; and (4) extend the time for adsorption and elution.
	3. Plasmid lost during strain storage	The bacterial cells have to be sub-cultured by transferring them to a new plate from primary culture before incubate the bacterial cells overnight for plasmid extraction.
	4. Poor cell lysis	Re-suspended the bacterial cells thoroughly in Buffer P1/RNase A.
	5. Incorrect reagent preparation	Dissolve precipitate in Buffer P2; added correct volume of ethanol to Buffer PW to keep the finally concentration of ethanol at 80%.
Genomic DNA contamination	1. Long culture time	Do not incubate cultures for more than 16 hr at 37°C.
	2. Inappropriate Lysis	Invert the tube gently after adding the Buffer P2; ensure the lysis time (starting from Buffer P2 added) does not exceed 5 min when extracting multiple samples.
Not suitable for down-steam applications	1. Salt pollution	Ensure that wash the pellet with Buffer PW2 twice.
	2. Ethanol pollution	Increase the centrifuge time to 5 min after the second washing with buffer PW2.
	3. Membrane falling off	Silicon membrane may fall off when centrifuge to elute plasmid. It is recommended to centrifuge the eluted DNA solution at 12,000 × g for another 1 min. Carefully collect the supernatant for down-stream use.