GeneKlean® Endotoxin-free Plasmid Miniprep Kit

Cat. Number: 21003EF-300

Storage: Store at Room Temperature.

Kit Contents

GeneKlean Plasmid Miniprep Kit	300Reactions
GeneKlean Column with 2 mL Collection Tube	300 pcs
Solution I	65mL
Solution II	65mL
Solution III	95mL
Wash Buffer I	125mL
Wash Buffer II	25mL×2
(Add Ethanol)	(100mL)×2
Elution Buffer	30mL
RNase A (50mg/mL)	150uL
This Datasheet	1 copy

Reagent Preparation

- Before use, add 96~100% ethanol to Wash Buffer II, **shaking before use**.
- If any precipitate forms in the solution II, it should be redissolved by warming it to 37°C before use.

Description

This kit provides a simple and efficient method for a small amount of endotoxin-free plasmid DNA preparation. DNA is selectively adsorbed by silica gel-based column and other impurities such as proteins, salts and nucleotides do not bind. Plasmid DNA can be eluted in a small volume of Tris buffer. When using the standard protocol, the entire process takes 15-20 minutes, without phenol / chloroform extraction or ethanol precipitation. Purified DNA can be used in any downstream applications such as sequencing, transformation, restriction enzyme digestion, and ligation.

Procedure for Preparation of Plasmid DNA

1. Pellet 1.5mL of overnight culture by centrifugation at 14,000g for 1 minutes. Drain the liquid completely.
2. Add 200uL of Solution I to the pellet, mix gently.
3. Add 200uL of Solution II to the mixture, mix gently by inverting the tube 10 times and then keep at RT for 2 minute. To prevent contamination from genomic DNA, do not vortex.

4. Add 300uL of Solution III, and mix gently. Then spin at 14,000g for 3 minutes.
5. Place spin column into a 2mL collection tube. Transfer supernatant (step 4) to the column.
6. Spin at 14,000g for 30 seconds discarding the flow-through in the tube. Then, place the spin column back into the same collection tube.
7. Add 400uL of Wash Buffer I to the column, and spin at 14,000g for 30 seconds. Discard the flow-through and place the column back into the same collection tube.
8. Add 600uL of Wash Buffer II(Ethanol added) to the column, and spin at 14,000g for 30 seconds. Discard the flow-through and place the column back into the same collection tub
9. Spin 14,000g again for 2 minutes to remove residual Wash Buffer II.
10. Transfer the column to a clean 1.5mL centrifuge tube.
11. Add 50uL of Elution Buffer to the center of the column for 2 minutes. Then, spin at 14,000g for 2 minute, get the plasmid DNA you wanted.

Note: Eluting plasmid DNA with 25uL of Elution Buffer two times will yield more DNA. More elution times are also possible. Store DNA in -20°C freezer.

Troubleshooting

Symptoms	Possible Causes	Suggestions
Low Plasmid DNA yields	Low copy number plasmid	Use more bacteria culture such as 3-5mL instead of 1.5mL.
	The cell pellet is not resuspended well.(1)	When more culture (3-5mL) is used, double the volume of Solution Ⅰ II and III
	The cell pellet is not resuspended well.(2)	The cell pellet can be resuspended by vortexing, but it may take longer time than scraping the base of the tube across the surface side of a centrifuge tube storage rack. This method may need some practice, but proved to be very efficient.
	Other elution solution is used	Elution Buffer is 2.5 mM Tris-HCl at pH 8.5. TE buffer at pH 8.0 or water can also be used. But yield will be slightly lower.
Small size nucleic acid band	RNA is co-purified with plasmid DNA	Check the storage time and conditions of reagents. Solution I with RNase A added will be stable for six months at 4-8°C.
High molecular weight DNA band	Genomic DNA contamination	When Solution Ⅱ is added, mix gently by inverting the tube 4-6 times, do not vortex.