GeneKlean® Plasmid Miniprep Kit

Cat. Number: 21003

Storage: Store at Room Temperature.
*RNase A: Store at -20°C before added to Solution I
**Solution I: After addition of RNase A, Solution I should be stored at 4°C for frequent use, or stored at -20°C if not used for a long period.

Sample: Up to 5 ml bacterial cells

Yield: up to 15 µg of plasmid

Kit Contents

Reagent

GeneKlean Plasmid Miniprep Kit	300 Reactions
GeneKlean Column with 2 mL Collection Tube	300
Solution I	90mL
Solution II	90mL
Solution III	90mL
Wash Solution	72mL
Elution Buffer	30mL
Boiled RNase A (10mg/mL) (Store at -20°C before added to Solution I)	900uL
This Datasheet	1 copy

Preparation

- Before use, add Boiled RNase A to Solution I. After addition of RNase A, Solution I should be stored at 4°C for frequent use, or stored at -20°C if not used for a long period.
- If any precipitate forms in the solution II, it should be redissolved by warming it to 37°C before use.
- Before use, add 96mL of 100% ethanol to 24mL of Wash Solution. If Wash Solution leaked during transportation, it is necessary to re-measure its volume, and determine the volume of ethanol added (ethanol:Wash Solution = 4:1).
- Elution Buffer is TE buffer at pH 8.0.

Description

This kit provides a simple and efficient method for a small amount of 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 8000g for 15 seconds. Drain the liquid completely. Attention: For low copy number plasmid, add 3-5mL of overnight culture and double the volume of Solution I, II and III.
2. Add 250uL of Solution I to the pellet, mix gently and keep on ice for 2 minutes.
3. Add 250uL of Solution II to the mixture, mix gently by inverting the tube 4-6 times and then keep at RT for 1 minute. To prevent contamination from genomic DNA, do not vortex.
4. Add 250uL of Solution III, and mix gently. Incubate at RT for 1 minute.
5. Spin at 12000g for 5 minutes(10 minutes for a better result).
6. Place spin column into a 2mL collection tube. Transfer supernatant (step 5) to the column. Let it stand for 2 minutes. Spin at 12000g for 1 minute.
7. Discard the flow-through in the tube. Add 500uL of Wash Solution to the column, and spin at 12000g for 1 minute.
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the collection tube. Spin at 12000g for additional 30seconds (1min for a better result) to remove residual Wash Solution.
10. Transfer the column to a clean 1.5mL centrifuge tube. Add 50uL of ddH2O (or Elution Buffer) to the center of the column and incubate at 37°C or 50°C for 2 minutes. Spin at 12000g for 1

minute. Note: Eluting plasmid DNA with 25uL of ddH2O (or Elution Buffer) two times will yield more DNA. More elution times are also possible. If the plasmid is used for PCR, ddH2O is recommended. 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.

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