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Plasmid DNA Extraction Maxiprep Kit

9K-006-0023s (1 prep)
9K-006-0023 (10 prep)
9K-006-0026 (30 prep)

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For Research Use Only

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Plasmid DNA Extraction Maxiprep Kit

Introduction

Feldan Plasmid DNA Extraction Maxiprep Kit is an excellent tool offering a rapid and economic method to purify plasmid DNA from bacteria cultures. This technology is based on alkaline lysis and purification by Anion-exchange chromatography. Compared with other harmful and time-consuming procedures such as phenol / chloroform extraction and ethanol precipitation, Feldan Plasmid DNA Extraction Kit shortens the handling time to about 2 hours. The high quality plasmid DNA can be used directly for any downstream applications.

Specifications

Sample Size	Yield	Handling Time
100~250 mL of bacteria culture for high copy plasmids	up to 500 µg for high-copy plasmids	About 2 hours
200~400 mL of bacteria culture for low copy plasmids		

Kit Contents

Component	9K-006-0023s, 1 Preps	9K-006-0023, 10 Preps	9K-006-0026 30 Preps
MAXI 1 Resuspension Solution	11ml	110ml	3x110ml
MAXI 2 Cell Lysis Solution	11ml	110ml	3x110ml
MAXI 3 Neutralization Solution	11ml	110ml	3x110ml
MAXI 4 Equilibration Solution	13.5ml	135ml	3x135ml
MAXI 5 Washing Solution	55ml	2x275ml	6x275ml
MAXI 6 Elution Solution	13.5ml	135ml	3x135ml
RNase A (50mg/ml)	22µl	220µl	3x220µl
MAXI Column	1 pc	10 pcs	30 pcs
Protocol	1	1	1

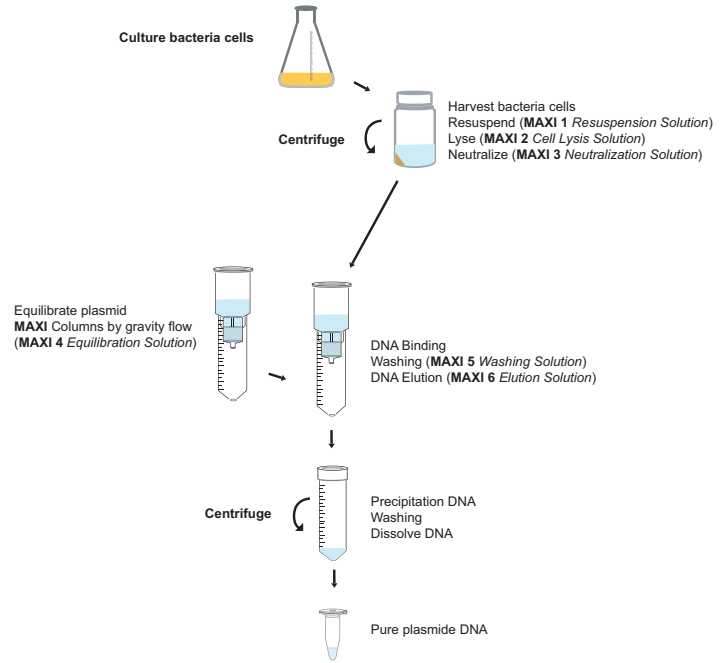
Important Notes

1. Solutions provided in this kit contain irritants, wear gloves and lab coat when handling.
2. Briefly spin RNase A tube to remove drops from the inside of the lid. Transfer tube content into MAXI 1 Resuspension Solution bottle. Add 250µL of MAXI 1 Resuspension Solution into RNase A tube, rinse tube inside and transfer back into MAXI 1 resuspension Solution bottle. Store at 4°C.
3. Check MAXI 2 Cell Lysis Solution before use. Warm MAXI 2 Cell Lysis Solution at 37°C if any pre-cipitation formed. Prevent vigorous shaking of the MAXI 2 Cell Lysis Solution.
4. To avoid acidification of MAXI 2 Cell Lysis Solution from CO2 in the air, close the bottle immediately after use.

Additional Material Required

1. 50 mL centrifuge tube.
2. Isopropanol
3. 70 % Ethanol

Brief Procedure



General Protocol

1. Harvest the bacterial culture by centrifugation at 6,000 x g for 15 minutes.
2. Add 10 ml of MAXI 1 Resuspension Solution (RNase A added) and resuspend the cell pellet by vortexing or pipetting.
3. Add 10 ml of MAXI 2 Cell Lysis Solution and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing of genomic DNA.
4. Incubate for 3 minutes at room temperature until lysate clears.
5. Add 10 mL of MAXI 3 Neutralization Solution and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing of genomic DNA.
6. Centrifuge at 15,000 x g for 20 minutes at 4 °C.
7. Transfer supernatant from step 6 in a new tube. Centrifuge at 15,000 x g for 20 minutes at 4°C.
8. Place a MAXI Column into a 50 mL centrifuge tube, add 10 mL of MAXI 4 Equilibration Solution to equilibrate the MAXI Column and allow the column to empty by gravity flow.
9. Transfer the supernatant from step 7 to the equilibrated MAXI Column, and allow the column to empty by gravity flow. Discard the filtrate.
10. Add 25 ml of MAXI 5 Washing Solution to wash the MAXI Column and allow the column to empty by gravity flow. Discard the filtrate.
11. Repeat step 10.
12. Place the MAXI Column into a clean 50 mL centrifuge tube (not provided) and add 12 mL of MAXI 6 Elution Solution to elute DNA by gravity flow.
13. Precipitate DNA by adding 9 mL of isopropanol to the eluted DNA from Step 12.
14. Mix gently and centrifuge at 20,000 g for 30 minutes at 4 °C.
15. Carefully remove the supernatant and wash the DNA pellet with 5 mL of room temperature 70% ethanol.
16. Centrifuge at 20,000 g for 10 minutes at 4°C.
17. Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.
18. Dissolve the DNA pellet in a suitable volume of 10mM tris pH 8.5 or ddH_2O

Troubleshooting

Low Yield

Bacterial cells were not lysed completely.

- Too many bacterial cells were used.
- After MAXI 3 *Neutralization Solution* addition, break up the precipitate by inverting.
- DNA pellet was lost after precipitation.
- DNA pellet was insufficiently redissolved.

Purified DNA doesn't perform well in downstream application.

RNA contamination

- Make sure that RNase A was has been added in MAXI 1 Resuspension Solution when first using. If RNase A added in MAXI 1 Resuspension Solution has expired, add additional RNase A.
- Too many bacterial cells were used, reduce the sample volume.
- Elution buffer contains EDTA.

Genomic DNA contamination

- Do not use overgrown bacterial culture.
- During MAXI 2 Cell Lysis Solution and MAXI 3 Neutralization Solution addition, mix gently to prevent genomic DNA shearing.
- Lysis time was too long (over 5 minutes).

Too much salt residual in DNA pellet

- Wash the DNA pellet twice with 70% ethanol