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BIO BASIC INC.

SK1249 (4 preps)
SK1250 (20 preps)

Plasmid DNA Extraction
MAXI Prep Kit

Version 5.1
ISO9001 Certified

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Introduction

Bio Basic Plasmid DNA Extraction Maxiprep Kit is an excellent tool offering a rapid and economic method to purify plasmid DNA from bacterial 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 application.

Specification

<table>
<tr><th>Sample Size</th><th>Yield</th><th>Handling Time</th></tr>
<tr><td>100-250mL of bacterial culture for high copy plasmids</td><td rowspan="2">Up to 500 µg for high copy-plasmids</td><td rowspan="2">About 2 hours</td></tr>
<tr><td>200-400 mL of bacterial culture for low copy plasmids</td></tr>
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Content

Component	SK1249 (4 preps)	SK1250 (20 preps)
MAXI 1 *Resuspension Solution*	55mL	2X110 mL
MAXI 2 *Cell Lysis Solution*	55 mL	2X110 mL
MAXI 3 *Neutralization Solution*	55 mL	2X110 mL
MAXI 4 *Equilibration Solution*	54 mL	2X135 mL
MAXI 5 *Washing Solution*	4X55 mL	4x275 mL
MAXI 6 *Elution Solution*	54 mL	2X135 mL
Liquid Endotoxin Eliminator	8 mL	40 mL
RNase A (50 mg/mL)	88 µL	2X220 µL
MAXI Column	4 pcs	20 pcs
User Manual	1	1

Additional Materials Required

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

Important Notes

1. Solutions provided in this kit contain irritants, wear gloves and lab coat when handling.

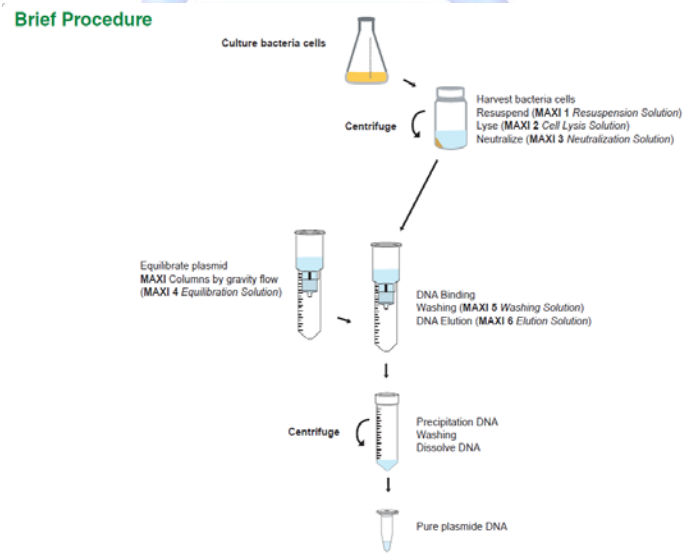
2. Briefly spin RNase A tube to remove drops from the inside of the lid. Transfer tube contents into **MAXI 1** *Resuspension Solution* bottle. Add 250 µL of **MAXI 1** *Resuspension Solution* into RNase A tube, rinse tube inside and transfer back into **MAXI 1** *Resuspension Solution* bottle. Store at 4°C.

3. Check **MAXI 2** *Cell Lysis Solution* before use. Warm **MAXI 2** *Cell Lysis Solution* at 37°C if any

precipitation formed. Prevent vigorous shaking of the **MAXI 2** *Cell Lysis Solution*.

4. To avoid acidification of **MAXI 2** *Cell Lysis Solution* from CO_2 in the air, close the bottle immediately after use.

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 pippetting.

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 imMaxiately 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. Discard the filtrate

9. Transfer the supernatant from step 7 to the equilibrated **MAXI** Column, and allow 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 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. Add 1.2 mL of pre-cold Liquid Endotoxin Eliminator to 12 ml of plasmid DNA solution. Incubate on ice for 10 minutes.

14. Incubate at 65ºC for 1-5 minutes.

15. Centrifuge at ≥15,000 *x g* for 5 minutes at room temperature.

16. Transfer the supernatant to a new endotoxin-free tube.

17. Repeat the procedure 2-3 times.

18. Add 9 mL of isopropanol to the solution, mix well by inverting 5 times. Incubate at -20°C for 20 minutes. Centrifuge at 15,000 x *g* for 5 minutes, discard the supernatant carefully.

19. Add 5 ml of pre-cooled 75% ethanol to the tube, mix well by inverting 10 times. Centrifuge at 15,000 x *g* for 1 minute, discard the supernatant.

20. Repeat the Step 19 once.

21. Air-dry the pellet at room temperature with the lid open for 2~5 minutes.

22. Add 50~200 µl of Endotoxin-Free water to dissolve DNA pellet. Keep at 4°C for a couple hours until DNA pellet is completely dissolved.

23. Purified plasmid DNA is ready to use, or store at -20 ºC freezer. For higher recovery yield, additional 2.5ml of Elution Buffer is added to the center part of the column and spin at 4,000 x g for 2 minutes. Measure OD260. Purified plasmid DNA is ready to use, or store at -20 ºC freezer.

Note: It is important to add the Elution Buffer into the center part. Pre-warm Elution Buffer at 55-80 ºC could increase elution efficiency. Two times elution is recommended.

Troubleshooting

Low yield

Bacterial cells were not lysed completely.

- Too many bacterial cells were used.
- After **MAXI 3** *Neutralization Solution*, 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 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 bacteria 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.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

Please visit www.biobasic.com

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