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BIO BASIC INC.

SK1249 (4 preps)
SK1250 (20 preps)

Plasmid DNA Extraction
MAXI Prep Kit

Version 6.0
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, Bio Basic 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

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

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 7 *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.

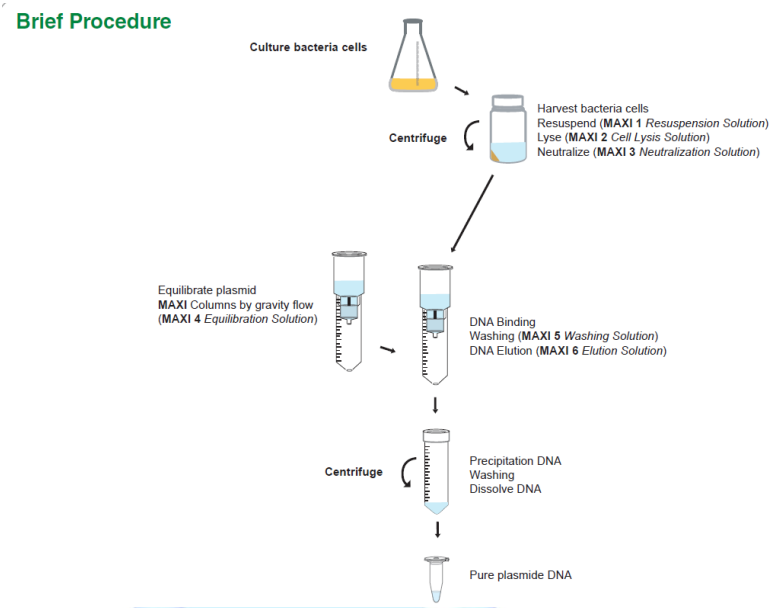
2. Briefly spin RNase A tube to remove drops from the inside of the lid. Transfer tube contents into **MAXI 1** *Resuspension Solution* bottle. Add 250 µL of **MAXI 1** *Resuspension Solution* into RNase A tube, rinse tube inside and transfer back into **MAXI 1** *Resuspension Solution* bottle. Store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

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 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. 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 7** Elution Solution to elute DNA by gravity flow.

13. Add 500 µl of pre-cold Liquid Endotoxin Eliminator to 12 ml of plasmid DNA solution. Incubate on Ice for 10 min.
14. Incubate at 65ºC for 1-5 minutes. (Solution may show some level of turbidity)
15. Centrifuge at ≥8000 *x g* (≥ 10,000 *rpm*) for 5 minutes at room temperature. (The solution is divided into two layers).

16. Transfer the supernatant to a new endotoxin-free tube.
17. Repeat step 13 to step 15 1-2 times.
18. Precipitate DNA by adding 9mL of isopropanol to the eluted DNA from Step 12.
19. Mix gently and centrifuge at 20,000 x g for 20 to 30 minutes at 4°C.
20. Carefully remove the supernatant and wash the DNA pellet with 5mL of room temperature 70% ethanol.
21. Centrifuge at 20,000 x g for 10 minutes at 4°C.
22. Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.
23. Dissolve the DNA pellet in suitable volume of 10mM tris pH8.5 or ddH_2O or MAXI 7 Buffer (endotoxin free).

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 the tube several times.
- 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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