MQ Plasmid DNA Maxi Extraction Kit

Cat No. PDE-M-001-20
Size: 20 Preps

MOLEQULE-ON®

Components	PDE-M-001-20 (20 Preps)
Maxi 1 (Resuspension Solution)	220 ml
Maxi 2 (Cell Lysis Solution)	220 ml
Maxi 3 (Neutralization Solution)	220 ml
Maxi 4 (Equilibration Solution)	270 ml
Maxi 5 (Washing Solution)	4 x 275 ml
Maxi 6 (Elution Solution)	270 ml
RNase A (50mg/ml)	440 ul
MQ Spin Column with 2.0ml Collection Tubes	20

Preparations

1. Solution 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. Add 250ul of Maxi 1 (Resuspension Solution) into RNase A tube. 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.

Storage

Maxi 1 should be store at 4°C. RNase A should be store at -20 °C, while all other reagents can be stored at room temperature.

Description

MQ Plasmid DNA Maxi Extraction 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. MQ Plasmid DNA Maxi Extraction Kit shorten the handling time to about 2 hours. The high quality plasmid DNA can be used directly for any downstream application.

Features

- MQ Plasmid DNA Maxi Extraction Kit procedure is simple, fast and efficient.
- It prepared high quality Plasmid DNA that can be used in any downstream applications such as sequencing, PCR, cloning, transformation or restriction digestions.
- The protocol is reproducible.
- For 100-250ml of bacterial culture for high copy plasmids and 200-400ml of bacterial culture for low copy plasmids, this kit yields up to 500 ug for high copy plasmids in about 2 hours.

Principle

The MQ Plasmid DNA Maxi Extraction Kit provides a simple and efficient method for plasmid DNA extraction. The plasmid DNA is selectively adsorbed in silica gel-based MQ column and other impurities such as proteins, salts, nucleotides, oligos (<40-mer) are washed away.

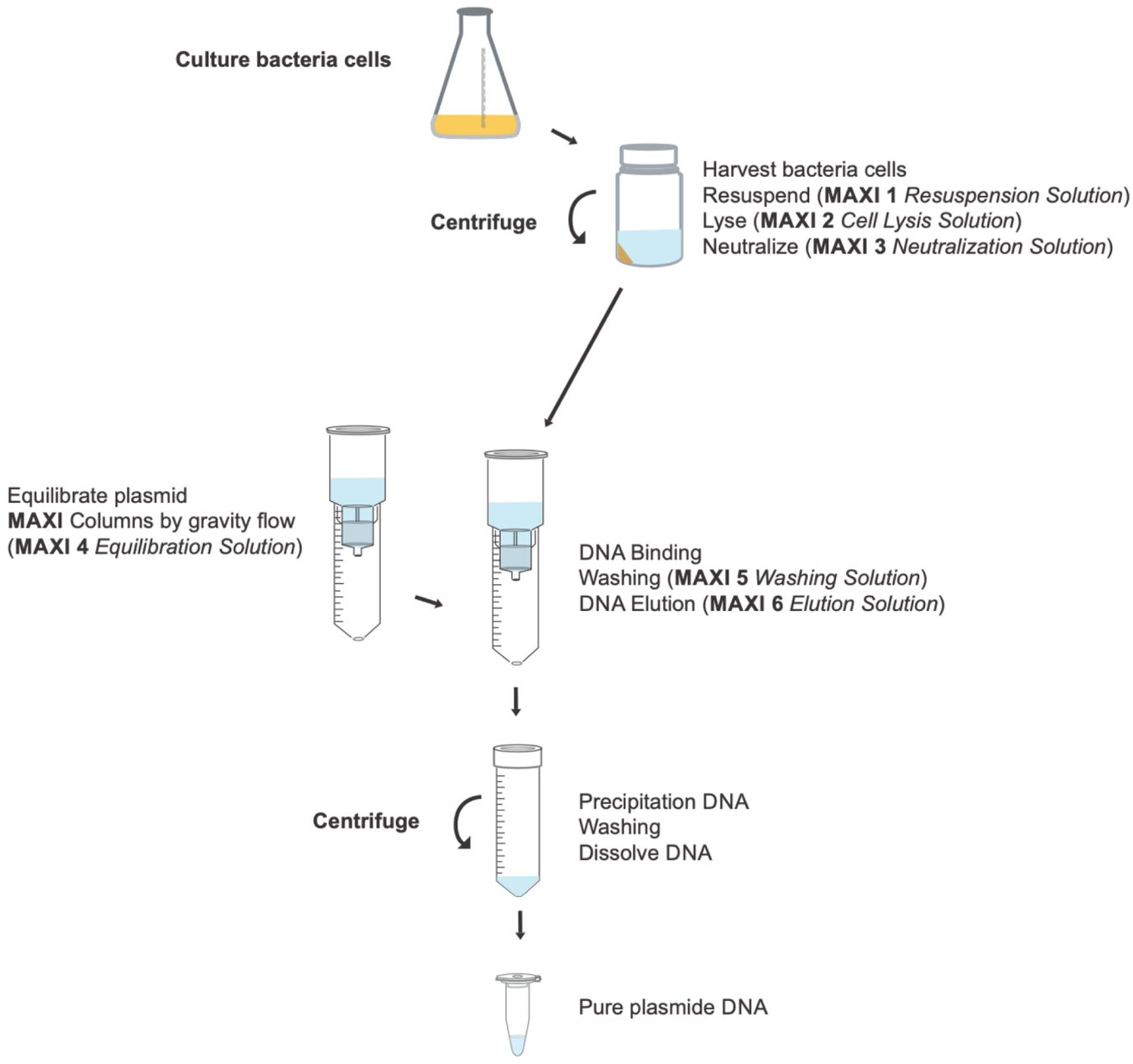
Additional Materials Required But Not Provided

- 50ml centrifuge tube
- Isopropanol
- 70% Ethanol

Procedure

1. Harvest the bacterial culture by centrifugation at 6,000 xg for 15 minutes.
2. Add 10ml of Maxi 1 (Resuspension Solution) (RNase A added) and resuspend the cell pellet by vortexing or pipetting.
3. Add 10ml of Maxi 2 (Cell Lysis Solution) and mix gentle 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 10ml 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 xg for 20 minutes at 4°C.
7. Transfer supernatant from step 6 in a new tube. Centrifuge at 15,000 xg for 20 minutes at 4°C.
8. Place a MQ Column into a 50ml centrifuge tube, add 10ml of Maxi 4 (Equilibration Solution) to equilibrate the MQ Column and allow the column to empty by gravity flow. Discard the filtrate.
9. Transfer the supernatant from step 7 to the equilibrated MQ Column, and allow column to empty gravity flow. Discard the filtrate.
10. Add 25ml of Maxi 5 (Washing Solution) to was the MQ Column and allow the column to empty by gravity flow. Discard the filtrate.
11. Repeat step 10.
12. Place MQ column into a clean 50ml centrifuge tube (not provided) and add 12ml 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 xg for 20-30 minutes at 4°C.
15. Carefully remove the supernatant and wash the DNA pellet with 5ml 70% ethanol at room temperature.
16. Centrifuge at 20,000 xg 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.

Brief Protocol



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 re-dissolved.

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.
- Too many bacterial cells were used, reduce the sample volume.
- Elution buffer contain 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.