MQ Plasmid DNA Extraction Kit

Cat No. PDE-M-002-100
Size: 100 Preparations

MOLEQULE-ON®

Component	Quantity (Volume)
Solution I	15ml x 2
Solution II	15ml x 2
Solution III	20ml x 2
Washing Buffer I*	24ml x 2
Washing Buffer II*	15ml x 2
RNase A	200ul x 2
Elution Buffer	15ml x 2
MQ Columns and Collection Tubes	100 pieces

Preparations

*Add Absolute Ethanol to the Washing Buffer before use. For 24ml Washing buffer I, add 16ml absolute ethanol before use. For 15ml Washing buffer II, add 60ml absolute ethanol before use.
Add 200ul of RNaseA to 15ml of Solution I before use, mix well, and store at 2-8℃ . Unless otherwise specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

Storage

Store at room temperature for 12 months. RNase A will be shipped as an accessory, store at -20℃ after receiving it.

Description

MQ Plasmid DNA Extraction Kit uses alkaline lysis method to lyse cells, and specifically extract plasmid DNA based on the principle that the centrifugal adsorption column specifically binds to the DNA in the solution under high salt conditions. The silicon matrix material used in the centrifugal adsorption column can adsorb DNA efficiently and specifically, and can remove impurity proteins and other organic compounds in cells to the greatest extent. The plasmid DNA extracted by this kit can be applied to various routine operations, including enzyme digestion, PCR, sequencing, ligation and transformation tests.

Features

- MQ Plasmid DNA 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.

Principle

The MQ Plasmid DNA Extraction Kit provides a simple and efficient method for plasmid DNA purification. 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. The plasmid DNA is then eluted off the column and can be used for any downstream application.

Procedure

1. Pellet 1-5ml of bacterial cultures by centrifugation for 1min at 12,000rpm in a microcentrifuge. Remove the supernatant as much as possible.
2. Completely resuspend the bacterial pellet in 250µl of Solution I (RNase A must be added before use). Complete resuspension of bacterial pellet is vital for obtaining good yields.
3. Add 250 µL Solution II. Invert and gently rotate the tube 6-8 times to obtain a clear lysate. A 2-3 minute incubation may be necessary, no more than 5 min to avoid destroying plasmid.

4. Add 350 μL SolutionⅢ , immediately and gently invert 6-8 times until a flocculent white precipitate forms. Centrifuge at 12,000rpm for 10 minutes.
Note: To avoid local precipitation, the solution should be mixed immediately after Solution Ⅲ is added. If there is a small white precipitate in the supernatant, please centrifugation again.
5. Carefully aspirate and transfer the cleared supernatant to a Adsorption Column. Incubate at room temperature for 2 min, centrifuge for 1min at 12,000rpm, discard the flow-through liquid, and re-use the collection tube in the next step.
6. Wash the Adsorption Column with 600μL Washing Buffer I, centrifuge at 12,000rpm for 1min, discard the flow-through and re-use the collection tube in the next step.
Note: Washing buffer must be diluted with absolute ethanol before use.
7. Wash the Adsorption Column with 700μL Washing Buffer II, centrifuge at 12,000rpm for 1min, discard the flow-through and re-use the collection tube in the next step.
Note: Washing buffer must be diluted with absolute ethanol before use.
8. Wash the Adsorption Column with 500μL Washing Buffer II, centrifuge at 12,000rpm for 1min, discard the flow-through and re-use the collection tube in the next step.
Note: Washing buffer must be diluted with absolute ethanol before use.
9. Centrifuge the empty column at 12,000rpm for 2min. Incubate at room temperature or 50℃ to dry the column. It is critical for removing Ethanol from the column.
10. Place column into a new clean centrifuge tube. Add 50-200μL Elution buffer onto the column matrix, incubate at room temperature for 2min. Centrifuge at 12,000rpm for 1min.
Note: Elution buffer should be preheated to 65℃ in advance in the water bath.
11. In order to increase DNA concentration, add the solution obtained from step 10 to the center of membrane again. Incubate at room temperature (15-25°C) for 2 min, and then centrifuge for 1 min at 12,000 rpm .

Important Points

1. Check for Solution Ⅱ, Solution Ⅲ before use, if the buffer appear turbid, dissolve it by warming at 37℃. Keep the cover of Solution Ⅱ, Solution Ⅲ, Washing Buffer I and Washing Buffer II tightly after use.
2. If the volume of eluted buffer is less than 50μL, it may affect recovery efficiency. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 8.0, below 7.0 will affect elution efficiency. DNA product should be stored at -20°C to avoid degradation.
3. Increase the volume of the Solution I, Solution Ⅱ and Solution Ⅲ , the time of absorbing and eluting if the plasmid is low copy or size>10kb.
4. DNA concentration and purity detection: The size of the genomic DNA fragments obtained is related to factors such as the storage time of the sample and the shearing force during the operation. The recovered DNA fragments can be tested for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak at OD_{260}, and an OD_{260} value of 1 is equivalent to approximately 50 μg/mL double-stranded DNA and 40 μg/mL single-stranded DNA. The OD_{260}/OD_{280} ratio should be 1.7-1.9. If elution buffer is not used for elution and deionized water is used, the ratio will be lower, because the pH value and the presence of ions will affect the light absorption value, but it does not mean that the purity is low.