MQ Plasmid DNA Mini Extraction Kit

Cat No. PDE-M-001-50 (50 Preps)
PDE-M-001-100 (100 Preps)
PDE-M-001-250 (250 Preps)

MOLEQULE-ON®

Components	PDE-M-001-50 (50 Preps)	PDE-M-001-100 (100 Preps)	PDE-M-001-250 (250 Preps)
Solution I	6 ml	12 ml	30 ml
Solution II	12 ml	24 ml	2 x 30 ml
Solution III	25 ml	2 x 25 ml	5 x 25 ml
VisualLyse	60 ul	120 ul	300 ul
Wash Solution*	20 ml	2 x 20 ml	2 x 40 ml
Elution Buffer	5 ml	10 ml	25 ml
RNase A Solution (10mg/ml)	120 ul	240 ul	600 ul
MQ Spin Column with 2.0ml Collection Tubes	50	100	250

Preparations

(A) Before the first use, add the RNase A Solution to the bottle containing Solution I and mix well. Solution I with RNase A should be stored at 4°C for frequent use and at -20°C for infrequent use.
(B) *Before the first use, add 80ml of 96-100% ethanol in 20ml of wash solution (160ml of 96-100% ethanol to 40ml Wash Solution).
(C) Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37°C.

Storage

Solution I should be store at 4°C. RNase A should be store at -20 °C, while all other reagents can be stored at room temperature. Kit can be stored for up to 24 months without showing any reduction in performance and quality.

Description

The MQ Plasmid Extraction system provides a fast, simple, and cost-effective plasmid miniprep method for routine molecular biology laboratory applications. This kit use a modified alkaline lysis method and silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Phenol extraction and ethanol precipitation are not required. It reduce time by extracting up to 20 µg high-purity plasmid or cosmid in 30 minutes or less. Plasmid DNA purified with MQ Plasmid Extraction Kits is immediately ready for use. The extracted plasmid DNA is suitable for a variety of experiments in molecular biology, including automated fluorescent DNA sequencing, restriction enzyme digestion, or transformation.

Features

- MQ Plasmid 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 Extraction Kit provides a simple and efficient method for mini 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. In order to maximize the recovery yield of plasmid DNA, a color indicator-VisualLyse is added to the buffer which prevents insufficient or over-lysis during lysis and neutralization step. The plasmid DNA is then eluted off the column and can be used for any downstream application.

Procedure

1. Centrifuge bacterial cells from 1.5 - 4.5 ml *E. coli* culture at 12,000 rpm for 2 minutes. Discard the supernatant.
   Note: If collect more than 1.5 ml bacterial cells, centrifuge and discard more times in the same tubes.

2. Add 100µl Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields. Keep for 1 minute.

1. Add 1 µl of VisualLyse to the mixture above. The addition of VisualLyse is an optional step.

1. Add 200 µl Solution II and mix thoroughly by inverting the tube 4–6 times. Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min. If VisualLyse has been added, the mixture will turn blue after addition of Solution II.

1. Add 350 µl Solution III and mix immediately and thoroughly by inverting the tube 4–6 times. Incubate at room temperature for 1 minute. A fluffy white material forms and lysate should become less viscous. If VisualLyse has been added, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless.

1. Centrifuge for 5 min at 12,000 rpm in a table-top microcentrifuge. A compact white pellet will form.

1. Apply the supernatants from above step to the spin column provided by decanting or pipetting. Be careful not to disturb the pellet and that no cellular debris is transferred to the spin column.

1. Centrifuge at 10,000 rpm for 2 minutes. Discard the flow-through.

1. Add 750µl Wash solution, and centrifuge at 10,000 rpm for 2 minutes. Discard the flow-through. Repeat Step 9 with another 750 µl Wash solution.

1. Place the spin column back into the same collection tube. Centrifuge the empty column at 13,000 rpm for 2 min to completely remove ethanol from the column.
   Note: It is important to dry the membrane of the spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

11. Place the column in a clean 1.5 ml microcentrifuge tube. Add 50 µl of Elution Buffer (Optional: pre-warm the buffer to 70–90°C will increase the DNA yield) to the center of the column membrane. Incubate at room temperature for 2-3 min, and centrifuge at 10,000 rpm for 2 minutes to elute the DNA.
    Note: Use smaller volume (minimum 30µl) of Elution Buffer will obtain higher concentration.

Optional: Put eluate back to the spin column to repeat elution once. This increases concentration of DNA about 10-15%.