Product Information

Plasmid Extraction Mini Kit

Catalog Number: P2035

Product Size: 100T, 200T

Application Scope: Plasmid extraction

Contents:

Component	100T	200T
A : U1 Buffer	25 mL	50 mL
B : U2 Buffer	25 mL	50 mL
C : U3 Buffer	35 mL	70 mL
D : W1 Buffer	45 mL	90 mL
E : Wash Buffer	20 mL	40 mL
F : Elution Buffer	15 mL	30 mL
G : Spin Column	100 pcs	200 pcs
H : Collection Tubes	100 pcs	200 pcs
I : RNase A	2.5 mg	5 mg

Storage

Store at room temperature. When stored as directed, product is stable for at least 12 months.

Description

US EVERBRIGHT's plasmid extraction kit is a kind of plasmid extraction product based on silica gel column, which can obtain high quality plasmid DNA in 25 min. Centrifugal spin column uses unique new materials to efficiently and specifically adsorb plasmid DNA. It can extract <15 kb plasmid DNA from the bacterial solution. Each column can bind up to 60 μg of plasmid DNA. The extracted plasmid can be used in downstream experiments, such as transformation, sequencing, PCR and digestion. The yield of plasmid extraction is related to the purity, the type and culture conditions of the host bacteria, the degree of cell lysis, plasmid stability, and the type of antibiotics added.

Protocol

Preparing the experiment

● Before the first use, add all RNase A to U1 Buffer and mix thoroughly.

● Before the first use, add an appropriate volume of absolute ethanol to the Wash Buffer. For example, add 80 mL of ethanol (96% to 100%) to 20 mL of Wash Buffer, or 200 mL of ethanol (96% to 100%) to 50 mL of Wash Buffer.

● If turbidity is found in U2 Buffer, it can be cleared by heating in 37 ℃ water bath.

● Prepare 1.5 mL centrifuge tube

Method

1. Transfer 1-3mL of well-grown bacterial solution to a centrifuge tube and the large amount can be divided into two transfers.

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2. Centrifuge at 11,000 × g for 1 min to precipitate bacteria and discard the supernatant.

3. Add 200 μL U1 Buffer (make sure that RNase A has been added), pipette and mix well to completely suspend the bacteria.

4. Add 200 μL U2 Buffer and mix gently for 5-10 times. Leave the cells at room temperature for 2-5 minutes to lyse the cells until the solution is clear. The lysis time should not exceed 5 minutes.

5. Add 300 μL U3 Buffer and mix with a pipette to avoid excessive white substance sticking to the tube wall.

6. Centrifuge at the maximum speed (~ 18,000 × g) for 5 minutes and collect the supernatant into the spin column. Be careful not to bring in white substances.

7. Place the spin column on the collection tube and centrifuge at 11,000 × g for 30 sec. Discard the filtrate and return to the collection tube.

8. Add 400 μL W1 Buffer to the spin column, centrifuge at 11,000 × g for 30 sec, discard the filtrate, and return to the collection tube.

9. Add 700 μL Wash Buffer (make sure ethanol has been added) to the spin column, centrifuge at 11,000 × g for 30 sec, discard the filtrate, and return to the collection tube.

10. Centrifuge at maximum speed (~ 18,000 × g) for 5 minutes to dry the spin column.

11. Place the spin column in a new 1.5 mL centrifuge tube.

12. Add 50 μL to 100 μL Elution Buffer or sterile water to the center of the column membrane and let stand for 1 minute.

13. Centrifuge at maximum speed (~ 18,000 × g) for 1 min to collect plasmid DNA, and store plasmid DNA at -20 ° C.

Notes

1. W1 Buffer contains irritating compounds. Wear latex gloves and glasses when handling. Avoid contamination of skin, eyes and clothing. Be careful not to inhale mouth and nose.

2. Bacterial breeding time should not exceed 16 h.

3. In step 10, make sure that the ethanol is completely removed, and allow it to stand for a few minutes at room temperature to allow it to evaporate.

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