

## Plasmid Extraction Mini Kit

Cat#:8084011

### [Product Name]

Plasmid Extraction Mini Kit

### [Model & Size]

| Product Name                | Cat No. | Size      | Component No. | Component Name   | Component Size | Quantity |
|-----------------------------|---------|-----------|---------------|------------------|----------------|----------|
| Plasmid Extraction Mini Kit | 8084011 | 100 T/Kit | 8084011-1     | P1 Buffer        | 25 mL          | 1 bottle |
|                             |         |           | 8084011-2     | P2 Buffer        | 25 mL          | 1 bottle |
|                             |         |           | 8084011-3     | P3 Buffer        | 35 mL          | 1 bottle |
|                             |         |           | 8084011-4     | W1 Buffer        | 45 mL          | 1 bottle |
|                             |         |           | 8084011-5     | Wash Buffer*     | 20 mL          | 1 bottle |
|                             |         |           | 8084011-6     | Elution Buffer   | 15 mL          | 1 bottle |
|                             |         |           | 8084011-7     | Spin Columns     | 100 pcs        | 1 pack   |
|                             |         |           | 8084011-8     | Collection Tubes | 100 pcs        | 1 pack   |
|                             |         |           | 8084011-9     | RNase A*         | 2.5 mg         | 1 bottle |

\* Add 80 mL ethanol (96%~100%) to Wash Buffer before first use;

\* Transfer all of RNase A to a P1 Buffer bottle before first use, refer to Precaution 2.

### [Product Introduction]

Plasmid Extraction Mini Kit is a silica gel column-based plasmid extraction product. It uses a unique new material in the centrifugal adsorption column to efficiently and specifically adsorb plasmid DNA, and high-quality plasmid DNA can be obtained within 25 min. The kit can extract plasmid DNA less than 15kb from bacterial broth. Each adsorption column could bind up to 60μg of plasmid DNA. The plasmid extracted using this kit can be used for downstream experiments, such as restriction enzyme digestion, transformation, PCR and sequencing. The yield and purity of plasmid extraction are related to the species of host bacteria, culture conditions, degree of cell lysis, stability of plasmid, types of antibiotics added and other factors.

### [Storage And Transportation]

RNase A is stored at -25°C~-15°C.

P1 Buffer after the addition of RNase A was stored at 2°C~8°C.

Other components were stored at room temperature (2°C~30°C) for 12 months.

Transport on blue ice.

### [Operating Instructions]

1. 1~3 ml of the bacterial solution from overnight cultures was transferred to a centrifuge tube, centrifuged at 11,000×g for 1 min, and the supernatant was discarded.

**Note: If the amount of bacterial fluid is large, it can be transferred several times, and the bacterial precipitate is collected into a centrifuge tube.**

2. Add 200 μL P1 Buffer, and use a pipette to gently blow to completely suspend the bacterial

precipitate.

**Note:** Ensure that RNase A has been added to P1 Buffer. If there is a bacterial block that is not thoroughly mixed, it will affect the lysis, resulting in low plasmid concentration and purity.

3. Add 200  $\mu$ L P2 Buffer, turn the centrifuge tube gently up and down for 5 to 10 times, stand at room temperature for 2 to 5 min to lyse the cells until the solution is clear, and the lye time should not exceed 5 min.

**Note:** Do not shake or vortex violently, so as not to break the genomic DNA and cause the extracted plasmid to be mixed with genomic DNA fragments. At this time, the bacterial solution becomes clear and viscous. If it does not become clear, it may be due to too many bacteria and incomplete lysis. The amount of bacteria should be reduced or the centrifuge tube should be turned over until the bacterial solution is clear.

4. Add 300  $\mu$ L P3 Buffer, and immediately turn it gently up and down for 5 to 10 times, and mix thoroughly to avoid too much white precipitate adhered to the tube wall.

**Note:** P3 Buffer should be mixed immediately after addition to avoid local precipitation.

5. Centrifuge at maximum speed ( $\leq 18,000\times g$ ) for 5 min.

6. Place the Spin Column on the Collection Tube and suck the supernatant obtained in step 5 into the Spin Column. After centrifugation at  $11,000\times g$  for 30 seconds, the filtrate was discarded and the Spin Column was returned to the Collection Tube.

**Note:** Do not inhale the white precipitate. If the volume of the supernatant was more than 800  $\mu$ L, it could be transferred to the Spin Column in multiple fractions.

7. Add 400  $\mu$ L W1 Buffer to the Spin Column, centrifuge at  $11,000\times g$  for 30 seconds, discard the filtrate, and put the Spin Column back into the Collection Tube.

8. Add 700  $\mu$ L Wash Buffer to Spin Column, centrifuge at  $11,000\times g$  for 30 seconds, discard the filtrate, and put the Spin Column back into the Collection Tube.

**Note:** Ensure that 80mL ethanol (96%-100%) has been added to Wash Buffer.

9. Centrifuge at maximum speed ( $\leq 18,000\times g$ ) for 3 min to dry the Spin Column and thoroughly remove residual ethanol.

**Note:** Residual ethanol can affect subsequent enzymatic reactions, such as restriction enzyme digestion, PCR, etc.

10. Put the Spin Column in a new 1.5 mL centrifuge tube, add 50  $\mu$ L~100  $\mu$ L Elution Buffer or ddH<sub>2</sub>O to the center of the Spin Column membrane, and let it stand for 1 minute.

11. The plasmid DNA was eluted by centrifugation at maximum speed ( $\leq 18,000\times g$ ) for 1 min. The eluted plasmid DNA was stored at  $-20^{\circ}\text{C}$ .

**Note:** The eluted solution can be sucked back into the Spin Column, left for 1 min, and centrifuged at  $18,000\times g$  for 1 min to increase the plasmid concentration. Elution Buffer or ddH<sub>2</sub>O was preheated to  $60^{\circ}\text{C}$  for better use.

### [Precaution]

1 Read the instructions carefully before using.

2. Before the first use, 0.5 mL P1 Buffer was added to the RNase A tube, vortexed and mixed. After a short centrifugation, all the mixed solution was transferred to the P1 Buffer bottle, mixed and stored at  $4^{\circ}\text{C}$ .

3. Before the first use, 80mL ethanol (96%-100%) should be added to Wash Buffer.

4. If P2 Buffer is found to be cloudy, it can be restored to clarity by heating in a  $37^{\circ}\text{C}$  water bath.

5. The centrifuge operation of this experiment can be completed at  $11,000\times g$ ~ $18,000\times g$ .

6. This product is limited to the scientific research of professionals. For your safety and health, please wear a laboratory coat and wear disposable gloves to operate.

**[Description Of Product Symbol]**

| Product Symbol                                                                    | Description                  | Product Symbol                                                                    | Description       |
|-----------------------------------------------------------------------------------|------------------------------|-----------------------------------------------------------------------------------|-------------------|
|  | Catalog Number               |  | Batch Code        |
|  | Date of Manufacture          |  | Manufacturer      |
|  | Keep away from sunlight      |  | Temperature limit |
|  | Consult instructions for use |  | Use-by Date       |

**[Company Information]**

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