5. Add 150 μL Buffer PB to the adsorption column and centrifuge at 13,000 rpm for 15 seconds.

Version: 05/2022

QuickPure Plasmid Mini Kit

Cat. No. : CW2619S (50 preps)
CW2619M (200 preps)

Shipping and Storage : Storage at room temperature (15-30°C).

Components

Component	CW2619S 50 preps	CW2619M 200 preps
Buffer L2	6 mL	25 mL
Buffer N3	20 mL	80 mL
Buffer PB	10 mL	35 mL
Buffer PW (concentrate)	6 mL	25 mL
Buffer EB	10 mL	30 mL
RNase A (10 mg/mL)	200 μL	800 μL
Spin Columns DM with Collection Tubes	50	200

Principle

This kit does not require centrifugation to collect bacteria and resuspend bacteria, directly add lysate Buffer L2 to the cultured bacterial solution, then neutralize and centrifuge through the column, the extracted plasmid can be up to 30 μg, and the entire extraction process does not exceed 10 minutes, and maximize the removal of other impurities such as protein and genome. The extracted plasmid DNA can be directly used for bacterial transformation, enzyme digestion, PCR, in vitro transcription, sequencing and other experiments.

Reagents to Be Supplied by User

96-100% ethanol

Preparation and Precautions before the Experiment

1. This kit can be stored in a dry, room temperature (15-30°C)) for 1 year. If you need to store for a longer time, the spin column can be placed at 2-8°C
2. Before the first use, add all the RNase A solution to Buffer N3, mix well, and store at 2-8°C.
3. Before the first use, add absolute ethanol to Buffer PW according to the instructions on the bottle label.
4. If there is precipitation in Buffer L2 before use, please place it in a 37°C water bath and keep mixing until the solution becomes clear before use.

Procedure

1. Take 600 μL bacterial culture into a 1.5 mL centrifuge tube (self-provided).
2. Add 100 μL Buffer L2 to the above centrifuge tube and mix upside down. The solution should turn from turbid to clear purple, indicating complete lysis. The lysis time should not exceed 2 minutes.
3. Add 350 μL Buffer N3 to the above centrifuge tube (please check whether RNaseA has been added), and immediately invert it up and down about 8-10 times to mix thoroughly. At this time, the solution should turn yellow completely and a yellow precipitate will form. Centrifuge at 13,000 rpm for 2-3 minutes.

4. Pour the supernatant obtained in step 3 into the prepared adsorption column (Spin Columns DM with CollectionTubes) slowly to avoid the precipitation entering the adsorption column.
5. Centrifuge at 13,000 rpm for 15 seconds, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
6. Add 150 μL Buffer PB to the adsorption column and centrifuge at 13,000 rpm for 15 seconds.
7. Add 400 μL Buffer PW to the adsorption column (please check whether absolute ethanol has been added), and centrifuge at 13,000 rpm for 1 minute.
8. Place the adsorption column in a new centrifuge tube (self-provided), add 30-100 μL of Buffer EB to the middle of the adsorption membrane, centrifuge at 13,000 rpm for 1 minute, collect the plasmid DNA, and store it at -20°C for a long time.

When the bacterial volume is >600 μL, the following steps are used:

1. This kit can extract up to 3 mL of bacterial solution. If the amount of bacterial solution extracted is more than 600 μL, it is necessary to centrifuge the bacterial solution exceeding 600 μL at 13,000 rpm for 30 seconds (to collect bacteria), discard the supernatant and then add 600 μL bacterial solution, thoroughly resuspend the bacterial cells at the bottom of the tube, and then proceed to the following operations.
2. Add 100 μL of Buffer L2 to the above centrifuge tube, and gently invert the solution up and down 10 times. If the solution is not clear, continue to invert and mix until the solution turns clear purple. The lysis time should not exceed 2 minutes. (If the solution is still turbid, it means that the amount of bacteria is too large, and the amount of bacteria needs to be reduced appropriately.)
3. Add 350 μL of Buffer N3 to the above centrifuge tube (please check whether RNaseA has been added), immediately invert it up and down and mix thoroughly until the purple solution completely turns yellow and a yellow precipitate forms, then proceed to the next step. Centrifuge at 13,000 rpm for 5 minutes.
4. Transfer the supernatant to a new centrifuge tube, add 200 μL isopropanol, invert up and down for several times, and transfer it to the adsorption column (Spin Columns DM with Collection Tubes) after mixing. Centrifuge through the column twice, centrifuge at 13,000 rpm for 15 seconds, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.