8. Place the adsorption column in a new collection tube, add 50-100 μL Buffer EB to the middle of the adsorption membrane, centrifuge at 13,000 rpm for 1 min, and collect the plasmid solution into the centrifuge tube. Store plasmids at -20°C.

Note:

1) In order to increase the recovery efficiency of the plasmid, the obtained solution can be added back to the adsorption column, placed at room temperature for 2-5 min, centrifuged at 13,000 rpm for 2 min, and the plasmid solution can be collected into a centrifuge tube.

2) When the plasmid copy number is low or >10 kb, Buffer EB is preheated in a water bath at 65-70°C, which can increase the extraction efficiency.

Version: 01/2024

GoldHi Plasmid Mini Kit

Cat. No. : CW2108M (200 preps)

Storage Condition: Store at room temperature (15-30°C).

Components

Component	CW2108M 200 preps
Buffer P1	60 mL
Buffer P2	60 mL
Buffer E3	60 mL
Buffer PW (concentrate)	25 mL
Buffer EB	30 mL
RNase A (10 mg/mL)	600 μL
Spin Columns DM with Collection Tubes	200

Introduction

This kit lyses cells by alkaline lysis method and uses a new type of silicon matrix membrane to efficiently and specifically bind plasmid DNA, which can extract 1-5 mL of bacterial liquid, and each adsorption column can adsorb up to 40 μg of plasmid DNA. A special buffer system is used to effectively remove impurities such as proteins to obtain plasmids with high yield, high purity and stable quality, which are suitable for cell transfection, DNA sequencing, PCR, in vitro transcription, bacterial transformation, and endonuclease digestion.

Reagents to be Supplied by user

100% ethanol
Isopropanol (100%)

Precautions

1. All components can be stored at room temperature (15-30°C) for 1 year, the adsorption column can be stored at 2-8°C for a longer time, and Buffer P1 with RNase A can be stored stably at 2-8°C for 6 months.
2. Add RNase A to Buffer P1 before use (add all the RNase A provided in the kit) and mix well, then store at 2-8°C. It needs to be placed at room temperature for a period of time before use, and then used after returning to room temperature.
3. Before the first use, add 100% ethanol to Buffer PW according to the instructions on the label of the reagent bottle.
4. Please check Buffer P2 and Buffer E3 for crystallization or precipitation before use. If there is, it can be clarified in a water bath at 37℃ for a few minutes.
5. Note that Buffer P2 and Buffer E3 contain irritating substances, please wear gloves to operate, and close the lid immediately after use.
6. The amount and purity of the extracted plasmid are related to the bacterial culture concentration, strain type, plasmid size, plasmid copy number and other factors.
7. The maximum volume of Spin Columns DM is 750 μL. If the sample volume is larger than 750 μL, it can be added in batches.

Protocol

1. Add 1-5 mL of overnight cultured bacterial solution to a centrifuge tube (self-provided), centrifuge at 13,000 rpm (~16,200×g) for 1 min to collect bacteria, and discard all supernatant.
2. Add 200 μL Buffer P1 (please check whether RNase A has been added) to the centrifuge tube with bacterial cell pellet, and mix well with a pipette or vortex shaker to suspend the bacterial pellet.
   Note: If the bacterial block is not thoroughly mixed, the lysis effect will be affected, resulting in lower extraction yield and purity.
3. Add 200 μL of Buffer P2 to the centrifuge tube, and mix by inversion for 8-10 times to fully lyse the cells. At this point the solution became clear and viscous.
   Note: Do not shake vigorously to avoid fragmentation of genomic DNA into the extracted plasmid. If the solution does not become clear, it indicates that the amount of bacteria may be too large and the lysis is not complete. At this time, the amount of bacteria should be reduced or the amount of P1, P2, E3 and isopropanol should be increased proportionally.
4. Add 200 μL Buffer E3 to the centrifuge tube, and immediately invert and mix for 8-10 times. At this time, a white flocculent precipitate appears and then centrifuge at 13,000 rpm for 5 min.
   Note: Buffer E3 should be mixed immediately after adding to avoid local precipitation.
5. After adding 260 μL isopropanol to the Spin Columns DM, which has been loaded into the collection tube, immediately add the supernatant collected in step 4, and mix by inversion.
   Note: The supernatant should be added immediately after the addition of isopropanol and mixed to avoid dropping the isopropanol into the collection tube after a long time. The maximum volume of the adsorption column is 750 μL. If the sample volume is greater than 750 μL, the isopropanol and supernatant can be collected in a centrifuge tube (self-provided), mixed well, and passed through the column in batches.
6. Centrifuge at 13,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
7. Add 400 μL Buffer PW to the adsorption column (please check whether 100% ethanol has been added), centrifuge at 13,000 rpm for 1 min, and discard the waste liquid in the collection tube.