9. Add 750 μL of Buffer PW to the column (please check whether 100% ethanol has been added before use), centrifuge at 13,000 rpm for 1 min, and discard the waste liquid in the collection tube.
10. Place the adsorbent column back into the collection tube and centrifuge at 13,000 rpm for 1 min.
    Note: The purpose of this step is to remove residual ethanol from the adsorbent column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
11. Place the adsorbent column in a new collection tube, add 50-100 μL Buffer EB to the middle part of the adsorbent membrane, leave it at room temperature for 2-5 min, centrifuge at 13,000 rpm for 2 min, and collect the plasmid solution into the centrifuge tube. -20°C to store the plasmid.
    Note: 1) In order to increase the recovery efficiency of the plasmid, the obtained solution can be re-added 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 the centrifuge tube.
    2)When the plasmid copy number is low or >10 kb, Buffer EB is preheated in water bath at 65-70°C, which can increase the extraction efficiency.

Version: 12/2023

SuperPlasmid Mini Kit

Cat. No. : CW2109S (50 preps)
Storage Condition: Store at room temperature (15-30°C).

Components

Component	CW2109S (50 preps)
Buffer P1	15 mL
Buffer P2	15 mL
Buffer E3	15 mL
Buffer PS	15 mL
Buffer PW (concentrate)	10 mL
Buffer EB	10 mL
RNase A (10 mg/mL)	150 μL
Spin Columns DM with Collection Tubes	50

Introduction

This kit provides a simple, fast and efficient extraction method of plasmid, suitable for the extraction of 1-5 mL bacterial fluid, based on the lysis of cells by alkaline lysis method, through the new silicone-based plasmid membrane efficiently and exclusively binding plasmid DNA, each adsorption column can adsorb a maximum of 40 μg of plasmid DNA, and effectively removes the contamination of genomic DNA, RNA, protein and other contaminants, easy and convenient to operate. The plasmid obtained from this kit is of high purity and stable quality, and can be directly used in biological experiments such as cell transfection, PCR, digestion, sequencing and ligation.

Reagents to be Supplied by user

100% ethanol, isopropanol

Precautions

1. All components can be stored stably in a dry, room temperature (15-30°C) environment for 1 year, the adsorption column can be stored at 2-8°C for a longer period of time, and Buffer P1 with RNase A added can be stored stably at 2-8°C for 6 months.
2. Before the first use, add all the RNase A solution into Buffer P1, mix well, and store it at 2-8°C, and leave it at room temperature for a period of time before use, and then use it after recovering to room temperature.
3. 100% ethanol should be added to Buffer PW according to the instructions on the reagent bottle label before first use.
4. Check Buffer P2 and Buffer E3 for crystallization or precipitation before use. If there is any crystallization or precipitation, the clarification can be restored by taking a water bath at 37°C for a few minutes (please do not shake Buffer P2 violently).
5. Be careful not to touch Buffer P2 and Buffer E3 directly, and tighten the lid immediately after use.
6. The amount and purity of extracted plasmid is related to the concentration of bacterial culture, strain type, plasmid size, plasmid copy number and other factors.

Protocol

1. Take 1-5 mL of overnight culture of bacteria, add it to a centrifuge tube (self-provided) , centrifuge at 13,000 rpm (~16,200 ×g) for 30 s to collect the bacteria, discarding as much of the supernatant as possible.
2. Add 250 μL Buffer P1 to the centrifuge tube that retains the bacterial precipitate (please check whether RNase A has been added before use) and suspend the bacterial precipitate by mixing thoroughly using a pipette or vortex shaker.
   Note: If the bacterial mass is not thoroughly mixed, it will affect the lysis effect and make the extraction amount and purity low.
3. Add 250 μL of Buffer P2 to the centrifuge tube, mix gently up and down 8-10 times to fully lyse the organisms, and leave at room temperature for 3-5 min. At this point the solution should become clear and viscous.
   Note: Mix gently, do not shake vigorously, as this may interrupt the genomic DNA and cause the extracted plasmid to be mixed with genomic DNA fragments. If the solution does not become clear, it suggests that the amount of bacteria may be too large and the lysis is not complete, and the amount of bacteria should be reduced (this step should not exceed 5 min).
4. Add 250 μL of Buffer E3 to the centrifuge tube and mix immediately by turning up and down 8-10 times, at which time a white flocculent precipitate appears, and leave it at room temperature for 5 min. Centrifuge at 13,000 rpm for 5 min, and aspirate the supernatant to be collected in a centrifuge tube (self-provided).
   Note: Buffer E3 should be mixed immediately after addition to avoid localized precipitation.
5. Add 225 μL of isopropanol to the filtrate and mix upside down.
6. Column balance step: Add 200 μL of Buffer PS to the Spin Columns DM with Collection Tubes, centrifuge at 13,000 rpm for 1 min, discard the waste liquid, and put the adsorbent column back into the collection tube.
7. Transfer the mixture of filtrate and isopropanol from step 5 to the Spin Columns DM with Collection Tubes.
8. Centrifuge at 13,000 rpm for 1 min, discard the waste liquid, and put the adsorbent column back into the collection tube.
   Note: The maximum volume of the column is 750 μL. If the sample volume is greater than 750 μL, add it in batches.