High purity plasmid miniaturization kit

Project number: P666142

Storage conditions: room temperature.

Products

individual parts making up a compound	/
Buffer P1	60ml
Buffer P2	60ml
Buffer N3	80ml
Buffer PB	35ml
Buffer PW (concentrate)	25ml
Buffer EB	30ml
RNase A (10mg/ml)	600 μl
Spin Columns DM with Collection Tubes	200

Products

This kit is suitable for extracting 1-5 ml of bacterial solution. Based on the lysis of cells by alkaline lysis method, it adopts a unique silica matrix membrane adsorption technology and reagent formulation, and efficiently and exclusively binds plasmid DNA in solution by centrifugal adsorption columns in a high-salt state, and each adsorption column can adsorb a maximum of 30 μg of plasmid DNA, and removes proteins, genomes, RNAs, and other impurities to the greatest extent possible. The plasmid DNA obtained can be directly used for cell transfection, PCR, digestion, sequencing, ligation and other biological experiments.

Self-contained reagent: anhydrous ethanol.

Pre-experiment Preparation and Important Notes

1. All components can be stably stored in dry, room temperature (15-30℃) environment for 1 year, the adsorption column can be stored at 2-8℃ for a longer period of time, and Buffer P1 with RNase A can be stably stored at 2-8℃ 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. Before use, leave it at room temperature for a period of time, and then use it after recovering to room temperature.
3. Anhydrous ethanol should be added to Buffer PW according to the instructions on the label of the reagent bottle before first use.
4. If precipitation is found in Buffer P2, Buffer N3, or Buffer PB before use, the clarification can be restored by water bath at 37℃ for a few minutes (please do not shake Buffer P2 violently).
5. Be careful not to touch Buffer P2, Buffer N3 and Buffer PB 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.

Procedure

1. Take 1-5 ml of the overnight culture and add it to a centrifuge tube (self-prepared), centrifuge for 30 seconds at 13,000 rpm (~16,200×g) to collect the bacterial precipitate, and discard the supernatant as much as possible.
2. Add 250 μl of Buffer P1 to the centrifuge tube with the bacterial precipitate (please check that RNase A has been added first), mix well using a pipette or vortex shaker, and suspend the bacterial precipitate.
Note: If the bacterial mass is not thoroughly mixed, it will affect the lysis effect, resulting in low extraction and purity.
3. Add 250μl of Buffer P2 to the centrifuge tube and mix gently up and down 4-6 times, mixing well to lyse the organisms, at which point the solution should become clear and viscous.
Note: Mix gently, do not shake vigorously to avoid interrupting the genomic DNA and causing the extracted plasmid to be mixed with genomic DNA fragments. This step should take no more than 5 minutes to avoid damage to the plasmid.
4. Add 350 μl of Buffer N3 to the centrifuge tube and immediately mix gently up and down for 8-10 times, mixing well so that a white flocculent precipitate should appear. centrifuge at 13,000 rpm for 5 minutes.
Note: Buffer N3 should be mixed immediately after addition to avoid localized precipitation.
5. Transfer the supernatant obtained in step 4 to the Spin Columns DM that have been loaded into the collection tube, centrifuge at 13,000 rpm for 30 seconds, pour off the waste liquid from the collection tube, and place the column back into the collection tube.
6. Add 150 μl Buffer PB to the adsorption column and centrifuge at 13,000 rpm for 30 seconds.
7. Add 400 μl Buffer PW to the adsorption column (please check that anhydrous ethanol has been added first), centrifuge at 13,000 rpm for 1 minute, and pour off the waste liquid in the collection tube.
8. Place the adsorbent column in a new centrifuge tube (supplied), add 50-100 μl Buffer EB to the middle of the adsorbent membrane, leave it at room temperature

for 2 minutes, centrifuge at 13,000 rpm for 1 minute, and collect the plasmid solution into the centrifuge tube. -The plasmid solution was collected into the centrifuge tube.

Note: 1) To increase the recovery efficiency of the plasmid, the resulting solution can be reintroduced into the adsorbent column, left at room temperature for 2 minutes, centrifuged at 13,000 rpm for 1 minute, and the plasmid solution collected into a centrifuge tube.

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