10. Add 2 mL Buffer PW (check first if anhydrous ethanol has been added) to the adsorption column, then centrifuge at 2500 ×g for 1 min. Discard the waste solution from the collection tube.
11. Repeat step 10.
12. Place the adsorption column back into the collection tube and centrifuge at 2500 ×g for 2 min. Discard the waste solution and leave the adsorption column to dry at room temperature for 5 min.

    Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can affect subsequent enzymatic reactions (digestion, PCR, etc.)
13. Place the column in a new 15 mL centrifuge tube and add 0.5-1 mL Endo-Free Buffer EB to the middle of the adsorbent membrane; leave at room temperature for 2-5 min, then centrifuge at 2500 ×g for 2 min and collect the plasmid solution into the centrifuge tube. Store the plasmids at -20°C.

    Note: 1) To increase the recovery efficiency of the plasmid, the solution obtained can be re-added to the adsorption column, left at room temperature for 2-5 min, centrifuged at 2500 ×g for 2 min and the plasmid solution collected into a centrifuge tube.

    2) When the plasmid is a low copy number plasmid or the size of the plasmid >10 kb, Endo-Free Buffer EB pre-warmed in a water bath at 65-70°C can increase extraction efficiency.

Version: 01/2021

GoldHi EndoFree Plasmid Midi Kit

Cat. No. : CW2581S (10 preps)

Shipping and Storage : Room temperature (15-30°C)

Components

Component	CW2581S 10 preps
Buffer P1	30 mL
Buffer P2	30 mL
Buffer E3	30 mL
Buffer PS	15 mL
Buffer PW (concentrate)	10 mL
Endo-Free Buffer EB	30 mL
RNase A (10 mg/mL)	600 μL
Endo-Remover FX	10
Plungers	10
Spin Columns DX with Collection Tubes	10
Centrifuge Tubes (15 mL)	10

Principle

This kit is designed for the efficient and rapid extraction of plasmids from 15-50 mL of bacterial broth. On the basis of lysing the cells by an alkaline lysis method, the unique silicon membrane binds plasmid DNA efficiently and specifically. Each adsorption column can adsorb up to 250 μg of plasmid DNA. While a special buffer system and endotoxin removal filter are used to effectively remove endotoxin, genomic DNA, RNA, protein and other impurities. The plasmids obtained from this kit are of high purity and stable quality. It can be used for cell transfection, as well as for DNA sequencing, PCR, in vitro transcription, endonuclease digestion and other experiments.

Reagents to Be Supplied by User

Anhydrous ethanol; isopropanol.

Important Points Before Starting

1. All components can be stored in a stable, dry, room temperature (15-30°C) environment for 1 year. The columns can be stored for longer at 2-8°C. Buffer P1 with RNase A is stable for 6 months at 2-8°C.
2. Before the first use, add the entire RNase A solution to Buffer P1, mix well and store at 2-8°C. Before use, it should be placed at room temperature for a period of time and then used after returning to room temperature.
3. Anhydrous ethanol should be added to Buffer PW before the first use according to the instructions on the reagent bottle label.
4. Check whether there is crystallization or precipitation of Buffer P2 and Buffer E3 before use. If crystallisation or precipitation is present, water bath at 37°C for several minutes can restore the clarification.
5. Take care not to touch Buffer P2 and Buffer E3 directly and close the lid tightly immediately after use.
6. The amount and purity of the extracted plasmids is related to the concentration of the bacterial culture, the type of strain, the size of the plasmid, the copy number of the plasmid and other factors.
7. Adsorption columns treated with Buffer PS are best used immediately to avoid the effects of prolonged storage.

Procedure

1. Take 15-50 mL overnight bacteria culture and add it into a centrifuge tube (self-provided). Centrifuge at 5000 ×g for 10 minutes to collect bacteria. Discard all the supernatant as much as possible.
2. Add 2.5 mL Buffer P1 (first check if RNase A has been added) to the centrifuge tube with pellets. Mix well by pipetting or vortex to suspend the pellet.
   Note: If the bacteria pellet is not thoroughly resuspended, the lysis effect will be affected, and the amount and purity of extracted DNA will be lower.
3. Add 2.5 mL Buffer P2 to the centrifuge tube and invert gently for 8-10 times. Then leave at room temperature for 3-5 minutes. At this point the solution should become clear and viscous.
   Note: Mix gently and do not vortex violently to avoid interrupting the genomic DNA, resulting in the extracted plasmid mixed with genomic DNA fragments. If the solution does not become clear, it may indicate that the amount of bacteria may be too large and the lysis is not complete. The amount of bacteria should be reduced.
4. Add 2.5 mL Buffer E3 to the centrifuge tube and invert immediately for 8-10 times. At this time, a white flocculent precipitate appears.
   Note: Buffer E3 should be mixed immediately after addition to avoid localised precipitation.
5. Install the cap of the filter (Endo-Remover FX), transfer the solution from step 4 to the filter. After the white flocculent precipitate floats on the upper layer of the solution, remove the cap of the filter and align it with a clean 15 mL centrifuge tube (self-provided), then slowly push the Plungers to filter and collect the filtrate in the tube.
6. Add 1/3 of the solution volume of isopropanol to the filtrate and mix by inverting.
7. Column equilibration: Add 1 mL Buffer PS to the Spin Columns DX packed with 15 mL centrifuge tube. Centrifuge at 2500 ×g for 2 minutes. Discard the waste liquid from the tube and put the column back into the tube.
8. Transfer the mixed solution in step 6 to the equilibrated adsorption column with a collection tube.
9. Centrifuge at 2500 ×g for 1 minute. Discard waste solution from the collection tube and put the adsorption column back into the collection tube.
   Note: The maximum volume of the adsorption column is 4 mL, so the solution obtained in step 8 is passed through the column in 2 passes.