8. Centrifuge at 13,000 rpm for 1 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

   Note: The maximum volume of the adsorption column is 750 μL, so the solution obtained in step 5 is passed through the column multiple times.

9. Add 750μL Buffer PW to the adsorption column (please first check whether anhydrous ethanol has been added), centrifuge at 13,000 rpm for 1 min, and drain the waste liquid from the collection tube.

10. Put the adsorption 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 adsorption column, which will affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.).

11. The adsorption column was placed in a new centrifuge tube (self-provided), and 100-200μL Endo-Free Buffer EB was added to the middle part of the adsorption film. The adsorption column was placed at room temperature for 2-5 min, and centrifuged at 13,000 rpm for 2 min. The plasmid was preserved at -20℃.

    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, and centrifuged at 13,000 rpm for 2 min. Endo-Free the Buffer EB in 65-70°C water bath heating, the appropriate extension of the adsorption and elution time, increase extraction efficiency. 2) plasmid copy number is low or > 10 KB, Endo - Free the Buffer EB in 65-70°C water bath heating, can increase the extraction efficiency.

Version: 03/2023

EndoFree Plasmid Midi Kit

Cat. No. : CW2105S (50 preps)

Storage Conditions: Room temperature (15-30°C).

Components

Component	CW2105S 50 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	10 mL
RNase A (10 mg/mL)	600 μ L
Endo-Remover FM with Collection Tubes	50
Spin Columns DL with Collection Tubes	50

Principle

Endotoxin is a common pollutant in plasmid extraction. Since eukaryotic cells are very sensitive to endotoxin, the presence of endotoxin in fruit plasmids can greatly reduce the transfection efficiency of eukaryotic cells. This kit provides a new method for simple, fast and efficient extraction of endotoxin-free plasmids. The extracted plasmids can remove endotoxin to the maximum extent and effectively remove the contamination of DNA, RNA, protein, etc.

This kit is suitable for the extraction of 5-15 mL bacterial solution. On the basis of alkaline lysis of cells, it uses a new silicon matrix membrane to bind plasmid DNA with high efficiency and specificity. Each adsorption column can adsorb plasmid DNA up to 100μg. The plasmid obtained by this kit is of high purity and stable quality, which is especially suitable for cell transfection. It can also be used for downstream experiments such as DNA sequencing, PCR, PCR-based mutation, in vitro transcription, transforming bacteria, and endonucase digestion.

Self-prepared reagent

Anhydrous ethanol, isopropyl alcohol.

Important things before the experiment

1. All components can be stably stored in dry, room temperature (15-30°C) for 1 year, longer storage can be placed at 2-8°C, and Buffer P1 added with RNase A can be stably stored at 2-8°C for 6 months.
2. Before the first use, all RNase A solution was added to Buffer P1, mixed and stored at 2-8°C. The solution should be kept at room temperature for a period of time before use, and then returned to room temperature for use.
3. Anhydrous ethanol should be added to the Buffer PW prior to first use as instructed on the label of the reagent bottle.
4. Please check Buffer P2 and Buffer E3 for crystallization or precipitation before use. If there is crystallization or precipitation, it can be cleared in a water bath at 37°C for a few min.
5. Be careful not to touch Buffer P2 and Buffer E3 directly, and close the lid immediately after use.
6. The amount and purity of extracted plasmid were related to bacterial culture concentration, strain type, plasmid size, plasmid copy number and other factors.

Operation procedure

1. Take 5-15 mL of the bacterial solution cultured overnight and add it into the centrifuge tube (prepared by oneself). Centrifuge at 13,000 rpm (~16,200×g) for 1 minute to collect bacteria, and try to absorb and discard all the supernatant.
2. Add 500 μL Buffer P1 (please check whether RNase A has been added first) into the centrifuge tube with bacterial precipitation, use a pipette or vortex oscillator to mix well and suspend bacterial precipitates.

   Note: If the fungus mass is not thoroughly mixed, the cracking effect will be affected, resulting in low extraction quantity and purity.
3. Add 500 μL Buffer P2 into the centrifuge tube, gently mix it upside down for 8-10 times to fully crack the bacteria, and place it at room temperature for 3-5 min. At this point, the solution becomes clear and viscous.

   Note: Mix gently, do not shake violently, so as not to break genomic DNA, resulting in the extracted plasmid mixed with genomic DNA fragments. If the solution does not become clear, it may indicate that the bacteria quantity is too large, the cracking is not complete, and the bacteria volume should be reduced.
4. Add 500 μL Buffer E3 into the centrifuge tube and mix it upside-down for 8-10 times immediately. At this time, white flocculent deposition appears and leave it at room temperature for 5 min. Centrifuge at 13,000 rpm for 5 min, absorb the supernatant and add the supernatant to the filter column (do-removerFM) (which has been loaded into the collection tube), centrifuge at 13,000 rpm for 1 min for filtration, transfer the filtrate from the collection tube to the centrifugal tube (self-provided).

   Note: 1) the Buffer E3 join immediately after blending, avoid to produce local precipitation.

   2) The maximum volume of the adsorption column is 750 μL, so please filter the supernatant twice and mix it in the same self-provided centrifugal tube.
5. Add 450 μL isopropyl alcohol to the filtrate and mix upside down.
6. Column balancing: 200μL Buffer PS was added into Spin Columns DL, which was loaded into the collection tube, and centrifuge at 13,000 rpm for 2 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
7. Transfer the filtrate and isopropyl alcohol mixture from Step 5 to the balanced adsorption column (loaded into the collection tube).