9. Add **10 mL Buffer PW** to the DNA-BindingTubes (please check if 100% ethanol has been added first) to remove the solution from the column.
10. Repeat step **9.**
11. Keep negative pressure suction for 10 min, remove the residual rinse liquid in the adsorption film, and dry the adsorption film. If more than 6 samples are pumped at one time, the suction time of negative pressure can be appropriately prolonged. After the adsorption film is thoroughly dried, turn off the negative pressure switch.

    Note: 1) The purpose of this step is to remove the residual ethanol in the column, and the residual of ethanol will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.)

    2) The usage time of vacuum is decided by the condition of the membrane. If the membrane is still wet, the column can be put into 65°C incubator for 30 minutes to dry it completely.
12. When the pressure returns to **0 mbar**, take off the column and place it in a new **50 mL** Centrifuge Tubes. Add **1-3 mL Endo-Free Buffer EB** to the middle of the membrane; Leave at room temperature for **2-5** min, then centrifuge at **12,000 ×g** for **5** min, and the plasmid is collected into the collection tube. Store the plasmid at -20°C.

    Note: 1) In order to increase the recovery efficiency of the plasmid, the elution can be added back to the column, leave at room temperature for 2-5 min, centrifuge at 12,000 ×g for 5 min, and the plasmid is 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 can be preheated in a water bath at 65-70°C to increase extraction efficiency.

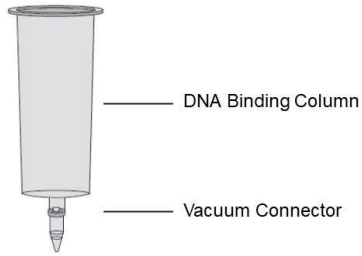

Diagram of connection between the column and connector

Version: 01/2024

GoldVac EndoFree Plasmid Maxi Kit

Cat. No. : CW2107S (2 preps); CW2107M (10 preps)

Storage Condition : Room temperature (15-30℃)

Components

Component	CW2107S	CW2107M
Buffer P1	30 mL	125 mL
Buffer P2	30 mL	125 mL
Buffer E3	30 mL	125 mL
Buffer PS	15 mL	30 mL
Buffer PW (concentrated)	10 mL	50 mL
Endo-Free Buffer EB	10 mL	30 mL
RNase A (10 mg/ml)	600 μL	2 mL
Endo-Remover FQ	2	10
DNA-Binding Tubes	2	10
VacConnectors	2	10
Centrifuge Tubes（50 mL）	2	20

Introduction

Endotoxin is a common pollutant in plasmid extraction. Eukaryotic cells are very sensitive to endotoxin, so if the plasmid contains endotoxin, it will greatly reduce the transfection efficiency of eukaryotic cells. This kit provides a new method for rapid and easy preparation of large-scale plasmids. 100-300 mL bacterial solution can be treated each time, and as many as 2 mg transfection grade plasmid DNA can be obtained. Using the vacuum device, multiple samples can be purified at the same time, and plasmid extraction can be completed in 45 minutes, which can effectively reduce the manual operation time. Special buffer system and endotoxin removal filter can effectively remove endotoxin, genomic DNA, RNA, protein and other impurities. The plasmid obtained by this kit has high purity and large extraction quantity, so it is especially suitable for cell transfection, and can also be used for DNA sequencing, PCR, in vitro transcription, endonuclease digestion and other experiments.

Reagents to be Supplied by user

100% ethanol; Isopropanol; Vacuum pump, waste liquid collection device, vacuum purification device.

Precautions

1. All components can be stored in a stable, dry, room temperature (15-30°C) environment for 1 year. The column can be stored at 2-8°C for longer storage time. Buffer P1 added with RNase A can be stored stably at 2-8°C for 6 months.
2. Add RNase A (all the RNase A provided in the kit) to Buffer P1 before use, mix well, and store at 2–8 °C. Before use, it should be left at room temperature for a period of time and then used after returning to room temperature.
3. 100% ethanol should be added to the Buffer PW before the first use according to the instructions on the bottle label.
4. Before use, please check whether Buffer P2 and Buffer E3 are crystallized or precipitated. If there is any crystallization or precipitation, it can be dissolved in a 37°C water bath for several minutes.
5. Note that Buffer P2 and Buffer E3 contain irritating substances. Wear gloves during operation. Close the lid immediately after use.
6. The column that has been treated with Buffer PS is best used immediately, because long storage time after treatment will affect the column's performance.
7. Please prepare the vacuum pump (CWE100), vacuum manifold(CWE200), and waste collection apparatus (CWE300). It is recommended to use the CWBIO products.

Vacuum Pump **Waste Collection** **Vacuum Manifold**

Protocol

1. Take **100-300 mL** overnight bacteria culture and transfer it to a centrifuge tube (self-prepared). Collect the bacteria by centrifugation at **12,000 ×g** for **2-3** min. Discard all the supernatant as much as possible.
2. Add **12 mL of Buffer P1** (please check if RNase A has been added) to the centrifuge tube with pellets. Mix well by pipetting or vortex to resuspend 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 **12 mL of Buffer P2** to the tube and invert gently for 8-10 times. Leave the tube to stand at room temperature for 3-5 min. 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 **12 mL of Buffer E3** to the tube and invert immediately for **8-10** times. A white flocculent precipitate should appear at this point. Allowed to stand at room temperature for 5 minutes. Pour the supernatant into **Endo-Remover FQ**, slowly push the handle to filter, and collect the flow-through in a clean **50 mL** centrifuge tube.
   Note: 1) Mixed immediately after Buffer E3 is added to avoid local precipitation.
   2) After adding Buffer E3, if there is excessive precipitation, it can be centrifuged at 12,000×g for 10 min, and then pour the supernatant solution into the Endo-Remover FQ.
5. Add **0.3** times volume of isopropanol to the flow-through and mix by inverting.
   Note: Adding too much isopropanol can easily lead to RNA
6. Connect the negative pressure device properly; Connect the **VacConnectors** to the **DNA-Binding Tubes** and insert them into the socket of the vacuum device.
   Note: Make sure that the connector and column are firmly connected to prevent air leakage.
7. Column balance: add 2 mL BufferPS to the DNA-Binding Tubes, turn on and adjust the negative pressure to -300~-700 mbar, and remove the solution from the column.
8. The mixed solution of filtrate and isopropanol in step 5 is transferred to a balanced adsorption column and the solution on the column is removed.