Version: 12/2023

GoldHi EndoFree Plasmid Maxi Kit

Cat. No. : CW2104S (2 preps)
CW2104M (10 preps)

Storage Condition: Store at room temperature (15-30°C).

Components

Component	CW2104S 2 preps	CW2104M 10 preps
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 PW	30 mL	125 mL
Endo-Free Buffer EB	10 mL	30 mL
RNase A (10 mg/mL)	600 µL	2 mL
Spin Columns DZ with Collection Tubes	2	10
Centrifuge Tubes (50 mL)	2	10

Introduction

Endotoxin is a common contaminant in plasmid extraction. Because eukaryotic cells are very sensitive to endotoxin, the transfection efficiency will be greatly reduced if endotoxin is contained in the plasmid. This kit provides a simple, rapid and efficient method for the extraction of endofree plasmids. Based on conventional alkaline lysis method, the new and unique silicon membrane binds plasmid DNA efficiently and specifically. At the same time by using a special buffer system and endotoxin-removal filters, impurities such as endotoxin, genomic DNA, RNA, proteins are effectively removed. 100-300 mL bacterial solution can be treated each time, and as many as 2 mg transfection grade plasmid DNA can be obtained. The whole extraction process takes only 50 minutes. The plasmid obtained by this kit has high purity and high yield, 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.

Precautions

1. All components can be stored stably in dry and 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.

10. Add 10 mL Endo-Free Buffer PW to the Spin Columns DZ and centrifuge at 12,000 ×g for 2 min. Discard the waste solution in the collection tube and put the Spin Columns DZ back into the collection tube.
11. Place the Spin Columns DZ back into the collection tube, centrifuge at 12,000 ×g for 5 min, discard the waste solution, and leave the column at room temperature for a few minutes to thoroughly dry the solution in the column.

    Note: The purpose of this step is to remove residual ethanol from the Spin Columns DZ; ethanol residue can interfere with subsequent enzymatic reactions (such as digestion, PCR).
12. The Spin Columns DZ is placed in a new centrifugal tube, 1-3 mL Endo-Free Buffer EB is added to the middle of the adsorption membrane, leave at room temperature for 2-5 min. Centrifuge at 12000 ×g for 5 min, and the plasmid will be collected into the centrifuge tube. The plasmid was preserved at -20°C.

    Note: 1) In order to increase the efficiency of plasmid recovery, the elution can be re-added to the Spin Columns DZ, leave at room temperature for 2-5 min. Centrifuge at 12000 ×g for 5 min, and the plasmid can be collected into the 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.

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 placed for 15-30 min and then the mixed liquid is passed through the column, and it is not recommended to leave it for more than 30 min.
7. The amount and purity of the extracted plasmid are related to factors such as bacterial culture concentration, strain type, plasmid size, and plasmid copy number.
8. The bacterial solution volume of plasmid extraction should not exceed the recommended volume in Table 1 and Table 2. The volume of P1, P2 and E3 solution extracted by high copy plasmid should all be 12 mL. For low copy plasmid extraction, the volume of P1, P2 and E3 solution should be adjusted according to the volume of bacterial liquid, which can be referred to Table 3:

Table 1

Maximum volume of bacterial solution for high copy plasmid						
Wet weight	ODV	OD_{600}=2	OD_{600}=4	OD_{600}=6	OD_{600}=8	OD_{600}=10
1.65 g	1000	500 mL	250 mL	166 mL	125 mL	100 mL

Table 2

Maximum volume of bacterial solution for low copy plasmid						
Wet weight	ODV	OD_{600}=2	OD_{600}=4	OD_{600}=6	OD_{600}=8	OD_{600}=10
2.0 g	1200	600 mL	300 mL	200 mL	150 mL	120 mL

Note: ODV = OD_{600} × V, V is the volume of bacterial liquid (mL), and OD600 is recommended to be 1-4.

9. The volume of lysate is recommended for low copy plasmids, as shown in the table below:

Table 3

Wet weight	2.0 g	1.6 g	1.3 g	1.0 g
Volume of bacterial liquid	300 mL	250 mL	200 mL	150 mL
Volume of P1, P2, E3	24 mL	20 mL	16 mL	12 mL

Protocol

1. Take 150 mL of the 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 pipette. Mix well by pipette or vortex to resuspend bacterial precipitation.
   Note: If the bacterial precipitation is not thoroughly mixed, it will affect the lysis effect and make the extraction volume and purity low. For low-copy plasmids, the volume of P1, P2 and E3 needs to be increased proportionally when the volume of the action bacterial solution is large, please refer to Table 3.
3. Add 12 mL of Buffer P2 to the tube and invert gently for 8-10 times, make the bacteria fully cleavage, let the tube stand at room temperature 5 min. At this point the solution should become clear and viscous.
   Note: Mix gently, do not shake violently, so as not to interrupt the genomic DNA, resulting in genomic DNA fragments mixed in the extracted plasmid. If the solution does not become clear, it is suggested that the volume of the bacteria may be too large and the lysis is not complete, so the volume of the bacteria should be reduced.

4. Perform column equilibration at this time: Add 2 mL Buffer PS to the Spin Columns DZ that has been loaded into the collection tube, centrifuge at 12,000 ×g for 2 min, discard the waste solution, and place the Spin Columns DZ back into the collection tube.
   Note: After the balance of the column in step 4 (Buffer PS passing through the column) until used in step 7 (the mixture of supernatant and isopropanol passing through the column), it is recommended to have a time interval of 15-30 min, which can improve the extraction yield.
5. Add 12 mL of Buffer E3 to the tube and invert immediately for 8-10 times. At this point a white flocculent precipitate appears, and let stand at room temperature for 5 min. Centrifuge at 12000 ×g for 10 min, transfer the supernatant to a clean centrifuge tube (self-provided), be careful not to bring into the precipitation.
   Note: Mixed immediately after Buffer E3 is added to avoid local precipitation.
6. Add 0.3 times the supernatant volume of isopropanol and mix upside down.
   Note: Adding too much isopropanol can easily lead to RNA contamination.
7. Transfer the mixed solution to the balanced Spin Columns DZ (loaded into the collection tube) in step 4. Centrifuge at 12000 ×g for 2 min, discard the waste solution, and place the Spin Columns DZ back into the collection tube.
   Note: The maximum volume of the column is 15 mL, so it will take multiple times to transfer the solution obtained in step 7. If the inclination angle of the centrifuge rotor is large, it is recommended that the volume of the solution added to the column each time does not exceed 10 mL to prevent leakage.
8. Add 10 mL Buffer PW (please check whether 100% ethanol has been added first) to the Spin Columns DZ, centrifuge at 12000 ×g for 2 min, discard the waste solution, and put the Spin Columns DZ back into the centrifuge tubes.
9. Repeat step 8.