3. If you need to improve the plasmid yield, you can appropriately increase the amount of bacterial liquid (when the volume of bacterial liquid exceeds the recommended amount in Table 1 and Table 2 above). At the same time, the volume of Buffer P1, P2 and E3 solution should be adjusted according to the volume of bacterial liquid. High copy plasmids can refer to Table 3, and low copy plasmids can refer to Table 4:

Table 3

ODV	1140	1030	915	800
Wet weight	2.0 g	1.8 g	1.6 g	1.4 g
Volume of P1, P2, E3	18 mL	16 mL	14 mL	12 mL

Table 4

ODV	1200	960	780	600
Wet weight	2.0 g	1.6 g	1.3 g	1.0 g
Volume of P1, P2, E3	24 mL	20 mL	16 mL	12 mL

Version: 02/2024

GoldHi EndoFree Plasmid Maxi Kit (Plus)

Cat. No. : CW2113M (10 preps)

Storage Conditions: All components can be stored stably in a dry, room temperature (15-30°C) environment for 1 year, and the adsorption column can be stored at 2-8°C for a longer period of time, and Buffer P1 with RNase A added can be stored stably at 2-8°C for 6 months.

Components

Component	CW2113M 10 preps
Buffer P1	125 mL
Buffer P2	125 mL
Buffer E3	125 mL
Buffer PS	30 mL
Buffer PW (concentrate)	50 mL
Endo-Free Buffer EB	30 mL
RNase A (10 mg/mL)	2 mL
Buffer ER	50 mL
CWBlue	3 mL
Spin Columns DZ with Collection Tubes	2×5 sets
Endo-Remover FQ	2×5 sets
Centrifuge Tubes (50 mL)	2×5 sets

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. And special endotoxin-free Buffer ER and Endo Remover FQ, which can effectively remove endotoxin, protein and other impurities. At the same time, it is equipped with special indicator CWBlue to ensure the efficient completion of plasmid extraction.

100-300 mL bacterial liquid can be treated each time, and the plasmid has high purity and large extraction quantity, and as many as 2 mg transfection grade plasmid DNA can be obtained. At the same time, it can also be used in DNA sequencing, PCR, in vitro transcription, endonuclease digestion and so on.

Reagents to be Supplied by user

100% ethanol, isopropanol.

Precautions

1. 100% ethanol should be added to the Buffer PW before the first use according to the instructions on the bottle label. Before the first use, add all the RNase A solution to Buffer P1 and mix well. Store at 2-8°C. It needs to be left at room temperature for a period of time before use and returned to room temperature before use.
2. Before use, please check whether Buffer P2, Buffer E3 and Buffer ER are crystallized or precipitated. If there is any crystallization or precipitation, it can be dissolved in a 37°C water bath for several minutes.
3. Note that Buffer P2 and Buffer E3 contain irritating substances. Wear gloves during operation. Close the lid immediately after use.
4. The column that has been treated with Buffer PS is placed for 15-30 minutes and then the mixed liquid is passed through the column, and it is not recommended to leave it for more than 30 minutes.

Attachment: Recommended volume of bacterial extract

The quantity and purity of extracted plasmid are related to the concentration of bacterial culture, the type of strain, the size of plasmid and the copy number of plasmid.

1. High copy plasmid: In Table 1, ODV = OD_{600} × V, V (mL) is the volume of bacterial liquid, that is, the amount of single treatment should not exceed the recommended ODV=800 in Table 1, and the corresponding wet weight of bacteria precipitation is about 1.4 g, which can also be used as a reference. Table 1 lists the Maximum volume of bacterial solution V (mL) corresponding to each bacterial liquid OD_{600}. When the volume of Buffer P1, P2 and E3 is 12 mL, the volume of bacterial liquid used at a single time should not exceed the recommended volume in Table 1.

Table 1

Maximum volume of bacterial solution for high copy plasmid, V (mL)						
Wet weight	ODV	OD_{600}=2	OD_{600}=4	OD_{600}=6	OD_{600}=8	OD_{600}=10
1.4 g	800	400 mL	200 mL	133 mL	100 mL	80 mL

2. Low copy plasmid: In Table 2, ODV = OD_{600} × V, V (mL) is the volume of bacterial liquid, that is, the amount of single treatment should not exceed the recommended ODV=600 in Table 2, and the corresponding wet weight of bacteria precipitation is about 1.0 g, which can also be used as a reference (If you need to increase the volume of bacterial solution or improve the yield, you need to increase the amount of solution according to Table 4). Table 2 lists the Maximum volume of bacterial solution V (mL) corresponding to each bacterial liquid OD_{600}. The volume of bacterial liquid used at a single time should not exceed the recommended volume in Table 2.

Table 2

Maximum volume of bacterial solution for low copy plasmid, V (mL)						
Wet weight	ODV	OD_{600}=2	OD_{600}=4	OD_{600}=6	OD_{600}=8	OD_{600}=10
1.0 g	600	300 mL	150mL	100mL	75 mL	60 mL

7. Step 5 after the completion of the water bath, immediately centrifuge 8000 rpm at room temperature for 10 min, when the bottom of the tube appears yellow oil phase, transfer the supernatant to a new centrifuge tube (self-provided), be careful not to absorb the bottom yellow oil phase.
8. Add isopropanol 0.3 times the volume of supernatant and mix upside down.
9. Transfer the mixed solution of the supernatant and isopropanol to the balanced Spin Columns DZ with Collection Tubes in step 6. Centrifuge 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 adsorption column is 15 mL, which can be divided into several times. If the inclination angle of the centrifuge rotor is large, it is suggested that the solution volume of the adsorption column should not exceed 10 mL to prevent liquid leakage.
10. Add 10 mL Buffer PW (please check whether 100% ethanol has been added first) to the adsorption column, centrifuge 12000 ×g for 2 min, discard the waste solution, and place the Spin Columns DZ back into the collection tube.
11. Repeat step 10.
12. Put the adsorption column back into the collection tube, centrifuge 12000 ×g for 5 min, discard the waste solution, and place the adsorption column at room temperature for 5 min to thoroughly dry the residual rinse liquid in the adsorption column.

    Note: The purpose of this step is to remove the residual ethanol from the adsorption column, which will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.).
13. The adsorption column is placed in a new centrifugal tube, 1-3 mL Endo-Free Buffer EB is added to the middle of the adsorption membrane, place at room temperature for 2-5 min, centrifuge 12000 ×g for 5 min, and the plasmid solution is collected into the centrifuge tube. The plasmid is preserved at -20°C.

    Note: 1) In order to increase the efficiency of plasmid recovery, the obtained solution can be re-added to the adsorption column, placed at room temperature for 2-5 min, centrifuge 12000 ×g for 5 min, and the plasmid solution can be collected into the centrifuge tube.

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

5. About CWBlue: CWBlue is an indicator. Please mix according to CWBlue: solution P1=1:100 and mix well. After adding Buffer P2 to fully mixing, the solution showed a uniform and clear blue, which indicated that the bacteria cells are lytic sufficiently; after adding Buffer E3 to fully mixing, the solution is colorless and transparent, and there is white flocculent precipitate floating in it, indicating that the neutralization and renaturation reaction is sufficient.

Protocol (Quick version steps)

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.

   Note: Please refer to the attachment for the volume of the extracted bacterial liquid.
2. Add 12 mL Buffer P1 (please check if RNase A has been added first) and 120 μL CWBlue to the centrifuge tube with bacterial precipitation. 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 yield and purity low.
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: 1) Mix gently, do not shake violently, so as not to interrupt the genomic DNA, resulting in genomic DNA fragments mixed in the extracted plasmid.

   2) 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.

   3) After adding Buffer P2 to fully mix, the solution will show uniform blue, and there is no obvious white flocs, which indicates that the bacteria cells are lytic fully.
4. 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.

   Note: Buffer E3 should be inverted and mixed immediately after addition to avoid localized precipitation. The solution is colorless and transparent, and loose bean flower-like white deposits float, which means neutralization is sufficient.

5. Column balance: Add 2 mL Buffer PS to Spin Columns DZ with Collection Tubes. Centrifuge at 12000 ×g for 2 min, discard the waste solution, and place the Spin Columns DZ back into the collection tube.

   Note: After column balance, it is recommended to rest for 15-30 min for step 8 (not more than 30 min), which can improve the performance of the adsorption column and improve the extraction.
6. After the completion of step 4, 12000 ×g centrifuge for 10 min, pour all the supernatant into the endotoxin removal filter (Endo-Remover FQ), try not to pour into the bulk precipitation, slowly push the handle to filter, and the filtrate is collected in a clean 50 mL centrifuge tube (self-provided).
7. Add 0.3 times the filtrate volume of isopropanol and mix upside down.
8. Transfer the mixed solution of the supernatant and isopropanol to the balanced Spin Columns DZ with Collection Tubes in step 5. Centrifuge 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 adsorption column is 15 mL, which can be divided into several times. If the inclination angle of the centrifuge rotor is large, it is suggested that the solution volume of the adsorption column should not exceed 10 mL to prevent liquid leakage.
9. Add 10 mL Buffer PW (please check whether 100% ethanol has been added first) to the adsorption column, centrifuge 12000 ×g for 2 min, discard the waste solution, and place the Spin Columns DZ back into the collection tube.
10. Repeat step 9.
11. Put the adsorption column back into the collection tube, centrifuge 12000 ×g for 5 min, discard the waste solution, and place the adsorption column at room temperature for 5 min to thoroughly dry the residual rinse liquid in the adsorption column.

    Note: The purpose of this step is to remove the residual ethanol from the adsorption column, which will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.).
12. The adsorption column is placed in a new centrifugal tube, 1-3 mL Endo-Free Buffer EB is added to the middle of the adsorption membrane, place at room temperature for 2-5 min, centrifuge 12000 ×g for 5 min, and the plasmid solution is collected into the centrifuge tube. The plasmid is preserved at -20°C.

    Note: 1) In order to increase the efficiency of plasmid recovery, the obtained solution can be re-added to the adsorption column, placed at room temperature for 2-5 min, centrifuge 12000 ×g for 5 min, and the plasmid solution can be collected into the centrifuge tube.

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

Protocol (Enhanced version of endotoxin removal)

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.

   Note: Please refer to the attachment for the volume of the extracted bacterial liquid.
2. Add 12 mL Buffer P1 (please check if RNase A has been added first) and 120 μL CWBlue to the centrifuge tube with bacterial precipitation. 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.
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: 1) Mix gently, do not shake violently, so as not to interrupt the genomic DNA, resulting in genomic DNA fragments mixed in the extracted plasmid.

   2) 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.

   3) After adding Buffer P2 to fully mix, the solution will show uniform blue, and there is no obvious white flocs, which indicates that the bacteria cells are lytic fully.
4. 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. 12000 ×g centrifuge for 10 min, pour all the supernatant into the endotoxin removal filter (Endo-Remover FQ), try not to pour into the bulk precipitation, slowly push the handle to filter, and the filtrate is collected in a clean 50 mL centrifuge tube (self-provided).

   Note: Buffer E3 should be inverted and mixed immediately after addition to avoid localized precipitation. The solution is colorless and transparent, and loose bean flower-like white deposits float, which means neutralization is sufficient.
5. Add 0.1 times the filtrate volume of Buffer ER, mix upside down. Ice bath 30 min, then 60°C water bath 10 min.
6. Column balance: Add 2 mL Buffer PS to Spin Columns DZ with Collection Tubes. Centrifuge at 12000 ×g for 2 min, discard the waste solution, and place the Spin Columns DZ back into the collection tube.

   Note: After column balance, it is recommended to rest for 15-30 min for step 9 (not more than 30 min), which can improve the performance of the adsorption column and improve the extraction.