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EndoFree Plasmid Maxi Kit

Product Number: PLK1301

Shipping and Storage

Room temperature (15-30℃).

Components

Component	PLK1301 2 preps	PLK1301 10preps
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 (concentrate)	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

Note:Self prepared reagents: anhydrous ethanol, isopropanol.

Description

Endotoxins are a common pollutant in plasmid extraction. Due to the high sensitivity of eukaryotic cells to endotoxins, the presence of endotoxins in plasmids can greatly reduce the transfection efficiency of eukaryotic cells. This kit provides a simple and efficient new method for extracting endotoxin free plasmids. Based on the alkaline lysis method for cell lysis, a unique silicon matrix membrane adsorption technology is used to efficiently and specifically bind plasmid DNA; At the same time, a special Buffer system and endotoxin removal Buffer are used to effectively remove impurities such as endotoxins, genomic DNA, RNA, proteins, etc. Each time, 100-300mL of bacterial solution can be processed to obtain up to 2mg of transfected plasmid DNA, and the entire extraction process only takes 50 minutes. The plasmid obtained from this kit has high purity and extraction capacity, making it particularly suitable for cell transfection. It can also be used for DNA sequencing, PCR, in vitro transcription, endonuclease digestion and other experiments.

Note

1. All components can be stably stored for 1 year in a dry, room temperature (15-30℃) environment. The adsorption column can be stored for a longer period of time at 2-8℃. Buffer P1 with RNase A added can be stably stored for 6 months at 2-8℃.
2. Add RNase A to Buffer P1 before use (add all RNase A provided in the reagent kit), mix well, and store at 2-8℃. Before use, it is necessary to leave it at room temperature for a period of time, and then use it after returning to room temperature.
3. Before the first use, anhydrous ethanol should be added to the Buffer PW according to the instructions on the reagent bottle label.
4. Before use, please check if there is any crystallization or precipitation in Buffer P2 and Buffer E3. If there is any crystallization or precipitation, you can take a water bath at 37℃ for a few minutes to restore clarity.
5. Note that Buffer P2 and Buffer E3 contain irritating substances. Please wear gloves when operating and immediately cover the lid after use.
6. The adsorption column treated with Buffer PS should be left for 15-30 minutes before passing the mixed solution through the column. The effect is good, and it is not recommended to leave it for more than 30 minutes.

7. The amount and purity of plasmid extraction are related to factors such as bacterial culture concentration, strain type, plasmid size, and plasmid copy number.
8. The amount of plasmid extraction bacterial solution should not exceed the recommended volume in Tables 1 and 2. The amount of P1, P2, and E3 solutions extracted from high copy plasmids is 12mL; For the extraction of low copy plasmids, the dosage of P1, P2, and E3 solutions should be adjusted according to the amount of bacterial solution, as shown in Table 3:

Table 1:

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

Table 2:

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

Note: ODV=OD_{600}×V. V is the amount of bacterial solution (mL); The recommended OD_{600} is 1-4.

9. It is recommended to use the volume of lysis solution 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
Bacterial liquid volume	300 mL	250 mL	200 mL	150 mL
Usage of P1, P2, E3	24 mL	20 mL	16 mL	12 mL

Note: Taking OD_{600}=4 as an example.

Protocol

1. Take 150mL of overnight cultured bacterial solution and add it to a centrifuge tube (provided by oneself). Centrifuge 12,000×g for 2-3 minutes to collect bacteria, and try to discard all the supernatant as much as possible.
2. Add 12mL Buffer P1 to the centrifuge tube containing bacterial sediment (please check if RNase A has been added first) and mix thoroughly with a pipette or vortex oscillator to suspend bacterial sediment.

   Note: If the bacterial blocks are not thoroughly mixed, it will affect the cracking effect, resulting in low extraction amount and purity. For low copy plasmids, it is necessary to increase the usage of P1, P2, and E3 proportionally when the amount of bacterial solution is large. Please refer to Table 3.
3. Add 12 mL of Buffer P2 to the centrifuge tube, gently invert and mix 8-10 times, allowing the bacterial cells to fully lyse. Let it stand at room temperature for 5 minutes. At this point, the solution should become clear and viscous.

   Note: Mix gently and do not shake vigorously to avoid interrupting genomic DNA and mixing genomic DNA fragments in the extracted plasmid. If the solution does not become clear, it indicates that the bacterial count may be too high and the lysis may not be complete. The bacterial count should be reduced.
4. At this point, perform column equilibrium: add 2 mL of Buffer PS to the Spin Columns DZ that has been loaded into the collection tube, centrifuge at 12,000×g for 2 minutes, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.

   Note: After the completion of column equilibrium in step 4 (Buffer PS passing through the column), it is recommended to have a time difference of 15-30 minutes until step 7 (supernatant and isopropanol mixture passing through the column), which can improve the extraction yield.
5. Add 12mL Buffer E3 to the centrifuge tube, immediately invert and mix 8-10 times. At this point, white flocculent precipitates appear, and let it stand at room temperature for 5 minutes. Centrifuge 12,000×g for 10 minutes and transfer the supernatant to a clean centrifuge tube (provided), being careful not to introduce sediment.

   Note: After adding Buffer E3, it should be mixed evenly immediately to avoid local precipitation.
6. Add 0.3 times the volume of the supernatant with isopropanol, invert and mix well.

   Note: Adding too much isopropanol can easily lead to RNA contamination.

7. Transfer the mixed solution of supernatant and isopropanol to the equilibrium adsorption column DZ (loaded into the collection tube) in step 4. Centrifuge 12,000×g for 2 minutes, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.

   Note: The maximum volume of the adsorption column is 15mL, so the solution obtained in step 7 is passed through the column multiple times. If the inclination angle of the centrifuge rotor is large, it is recommended to add a solution volume of no more than 10mL to the adsorption column to prevent liquid leakage.

8. Add 10mL of Buffer PW to the adsorption column (please check if anhydrous ethanol has been added first), centrifuge 12,000×g for 2 minutes, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.
9. Repeat step 8.
10. Add 10mL Endo Free Buffer PW to the adsorption column, centrifuge 12,000×g for 2 minutes, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.
11. Place the adsorption column back into the collection pipe, centrifuge at 12,000×g for 5 minutes, discard the waste liquid, and place the adsorption column at room temperature for a few minutes to thoroughly dry the residual rinsing solution in the column.

    Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can affect subsequent enzymatic reactions (such as enzyme digestion, PCR, etc.).

12. Place the adsorption column in a new centrifuge tube, add 1-3 mL Endo Free Buffer EB to the middle of the adsorption membrane, let it stand at room temperature for 2-5 minutes, centrifuge at 12,000×g for 5 minutes, and collect the plasmid solution into the centrifuge tube- Store the plasmid at 20℃.

    Note: 1) To increase the recovery efficiency of plasmids, the obtained solution can be added back to the adsorption column and left at room temperature for 2-5 minutes. Centrifuge 12,000×g for 5 minutes and collect the plasmid solution into a centrifuge tube.

    2) When the plasmid copy number is low or>10kb, preheating the Endo Free Buffer EB in a water bath at 65-70℃ can increase the extraction efficiency.