Endotoxin-free Plasmid Mass Extraction Kit

Cat#：8024031

[Product Component]

Product Name	Cat No.	Size	Component No.	Component Name	Component Size	Quantity
Endotoxin-free Plasmid Mass Extraction kit	8024031	10 T/Kit	8024031-1	Maxiprep column	10	2 package
			8024031-2	RNase A	270 μL	1 bottle
			8024031-3	Buffer S1	115 mL	1 bottle
			8024031-4	Buffer S2	115 mL	1 bottle
			8024031-5	Buffer S3K	115 mL	1 bottle
			8024031-6	Buffer B	115 mL	1 bottle
			8024031-7	Buffer W1	160 mL	1 bottle
			8024031-8	Buffer W2 concentrate	66 mL	1 bottle
			8024031-9	ET-free water (70%Ethanol)	7.5 mL	1 bottle
			8024031-10	Eluent A	30 mL	1 bottle
			8024031-11	Buffer ETR	50 mL	1 bottle
			8024031-12	Buffer PF	13 mL	1 bottle

[Product Description]

The method used in this kit is the SDS alkaline lysis method, which selectively adsorbs DNA through a DNA preparation membrane. It can extract up to 500 μg of high-purity plasmid DNA from 80 mL~200 mL of bacterial culture. At the same time, the solution Buffer ETR and Buffer PF are used to effectively remove the remaining endotoxin in the DNA, and the endotoxin level is ≤0.1 EU/μg.

[Storage conditions]

RNase A: 50 mg/mL, which can be stored at room temperature for 6 months, or at -25~-15℃ for long-term storage.
Buffer S1 should be stored at 2~8℃ after adding RNase A.
Other components should be stored at room temperature (2℃~30℃) and are valid for 12 months.

[Note]

(1) Add all RNase A to Buffer S1 and store at 2~8℃;
(2) Add the specified volume of absolute ethanol to Buffer W2 concentrate;
(3) Add the specified volume of absolute ethanol to ET-free water (70% Ethanol), and pre-cool at -25~-20℃ before use;
(4) Check whether there is precipitation in Buffer S2. If precipitation occurs, it should be dissolved in a 37℃ warm bath and cooled to room temperature before use;
(5) Buffer S3K, Buffer B and Buffer ETR should be pre-cooled at 2~8℃ before use.

[Protocol]

(1) Take 80~200 mL of bacterial liquid cultured overnight (if rich culture medium is used, the bacterial liquid volume should be reduced by half or less), centrifuge at 3,000 × g for 8 minutes, and discard the supernatant. Place the centrifuge tube upside down on paper towels for 1 minute and remove the supernatant;
*The OD_{600} of the bacterial liquid cultured overnight is between 2.0 and 4.0. If the OD_{600} of the bacterial liquid is >4.0, the amount of bacteria need to be reduced.
(2) Add 10 mL Buffer S1 to suspend the bacterial pellet, shake and mix well, and there should be no small bacterial clumps left;
(3) Add 10 mL Buffer S2 and mix gently by turning it up and down to fully lyse the bacteria until a clear solution is formed. This step should not exceed 5 minutes;
*Buffer S2 should be tightly capped immediately after use to prevent CO_2 in the air from neutralizing the NaOH in Buffer S2 and reducing the lysis efficiency.
(4) Add 10 mL of 4℃ pre-cooled Buffer S3K, gently turn up and down and mix until the solution is uniform in color and forms a tight agglomerate. Let stand at room temperature for 5 minutes, then centrifuge at 8,000 × g for 10 minutes at 4℃;
*Mix immediately after adding Buffer S3K to avoid the formation of local clumps.
(5) Transfer the supernatant in step (4) into a new 50 mL centrifuge tube (self-prepared), add 10 mL of 4℃ pre-cooled Bufer B, and mix gently by turning it up and down 10 times;
You can choose centrifugation method or negative pressure method for steps (6) to (8).
A. Centrifugal method:
(A6) Place the large-volume preparation tube into a 50 mL centrifuge tube, aspirate the mixed solution in step (5), transfer to the large-volume preparation tube, and centrifuge at ≥6,000 × g for 5 minutes;
(A7) Discard the filtrate, add 12 mL Buffer W1 to the preparation tube, and centrifuge at ≥6,000 × g for 5 minutes;
(A8) Discard the filtrate, add 14 mL Buffer W2 to the preparation tube, and centrifuge at ≥6,000 × g for 5 minutes;
B. Negative pressure method:
(B6) Correctly connect the negative pressure device and insert a large number of preparation tubes into the socket of the negative pressure device. Aspirate the mixed solution in step (5), transfer it to a large number of preparation tubes, turn on the power, adjust the negative pressure to -25 ~ -30 inches of mercury, and slowly suck out the solution in the tube;
(B7) Maintain negative pressure, add 12 mL Buffer W1, and aspirate the medium solution;
(B8) Maintain negative pressure, add 14 mL Buffer W2, and aspirate the medium solution;
(9) Add 4 mL of Buffer W2 to the large volume preparation tube, and centrifuge at ≥6,000×g for 5 minutes;
(10) Discard the filtrate, take out the preparation tube, place it in another clean 50 mL centrifuge tube, add 2 mL EluentA to the center of the membrane of the preparation tube, let stand at room temperature for 5 minutes, and centrifuge at ≥6,000×g for 5 minutes to collect plasmid DNA.
*Heating Eluent A to 65℃ will increase elution efficiency.
(11) Discard the preparation tube and add to the filtrate

[Precautions]

1. Excessive bacteria will affect bacterial lysis and release of plasmid DNA, resulting in excessive endotoxin content;
2. The operations of steps (3) and (4) must be gentle. Vigorous shaking will cause contamination of genomic DNA; but the mixing must be sufficient, otherwise the yield will be affected;
3. When adding Buffer S3K, the protein and genomic DNA form a viscous white flocculent precipitate, which must be fully mixed so that the middle of the agglomerate is fully neutralized and coagulated;
4. Buffer S2, Buffer S3K, Buffer B and Buffer W1 contain irritating compounds. Wear latex gloves and protective glasses when operating to avoid contamination of skin, eyes and clothing, and be careful not to inhale into the mouth and nose. If the skin or eyes are contaminated, rinse immediately with plenty of water or saline and seek medical consultation if necessary.

[Description of Product Symbol]

Product Symbol	Description	Product Symbol	Description
REF	Catalog Number	LOT	Product Batch Code
	Date of Manufacture		Expiration Date
	Manufacturer		Temperature limitation
	Consult instructions for use		Store away light

[Company Information]

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