Endotoxin-Free Plasmid Midi Extraction Kit

Cat#：8024021

[Product Component]

Product Name	Cat No.	Size	Component No.	Component Name	Component Size	Quantity
Endotoxin-Free Plasmid Midi Extraction Kit	8024021	10 T/Kit	8024021-1	Midiprep column	10	2 package
			8024021-2	1.5 mL Microfuge tube	30	1 package
			8024021-3	Plastic wrench	1	1
			8024021-4	RNase A	120 μL	1 bottle
			8024021-5	Buffer S1	55 mL	1 bottle
			8024021-6	Buffer S2	55 mL	1 bottle
			8024021-7	Buffer S3K	55 mL	1 bottle
			8024021-8	Buffer B	55 mL	1 bottle
			8024021-9	Buffer W1	80 mL	1 bottle
			8024021-10	Buffer W2 concentrate	36 mL	1 bottle
			8024021-11	ET-free water (70%Ethanol)	3.6 mL	1 bottle
			8024021-12	Eluent A	8 mL	1 bottle
			8024021-13	Buffer ETR	4 mL	1 bottle
			8024021-14	Buffer PF	1 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 100 μg of high-purity plasmid DNA from 30 mL~50 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.

[Operating steps]
(1) Take 30~50 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 4.5 mL Buffer S1 to suspend the bacterial pellet, shake and mix well, and there should be no small bacterial clumps left;
(3) Add 4.5 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 4.5 mL of 4℃ pre-cooled Buffer S3K and mix gently by turning it up and down until the solution is uniform in color and forms a tight agglomerate. Stand at room temperature for 5 minutes, then centrifuge at ≥6,000×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 4.5 mL of 4℃ pre-cooled Bufer B, and mix gently by turning it up and down 10 times;
Steps (6) to (8) are the negative pressure method:
(6) 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;
(7) Maintain negative pressure, add 7 mL Buffer W1, and aspirate the medium solution;
(8) Maintain negative pressure, add 8 mL Buffer W2, and aspirate the medium solution;
(9) Use a plastic wrench to remove the preparation tube head containing plasmid at the bottom of the medium-volume preparation tube, place it in a clean 1.5 mL centrifuge tube (provided in the Kit), add 0.3 mL Buffer W2, and centrifuge at 12,000×g for 2 minutes;
(10) Place the tip of the preparation tube into another clean 1.5 mL centrifuge tube (provided in the Kit), add 350 μL EluentA to the center of the membrane of the preparation tube, stand at room temperature for 1 minutes, and centrifuge at 12,000×g for 1 minutes to collect the plasmid DNA.
*Heating Eluent A to 65℃ will increase elution efficiency.
(11) Discard the preparation tube, add 350 μL of pre-cooled Buffer ETR to the filtrate, and mix evenly;
*If Buffer ETR is turbid, stand on ice until the solution becomes clear; if stratification occurs or the plasmid DNA is fragmented after endotoxin removal, the ETR reagent bottle could be reused by heating at 65℃ for 2 hours and pre-cooling at 2~8℃.
(12) Add 88 μL Buffer PF and mix evenly. The solution may appear slightly turbid, which is normal;
(13) Incubate at 56℃ for 2~5 minutes, and centrifuge at 12,000×g for 2 minutes at room temperature;
*After incubation, a large amount of milky white precipitate should appear in the solution, otherwise the incubation time will be extended.
*The solution may stratify after incubation and it is normal.

(14) Take the colorless upper phase into a 1.5 mL centrifuge tube (provided in the Kit), add 0.8 times the volume of isopropyl alcohol, mix evenly, stand at room temperature for 10 minutes, and centrifuge at 12,000×g for 10 minutes;
(15) Discard the supernatant as much as possible, add 1 mL of pre-cooled ET-free water (70% Ethanol), wash the precipitate, and centrifuge at 12,000×g for 5 minutes;
(16) Discard the supernatant as much as possible and dry it in a clean bench for 5~10 minutes;
(17) Add 150~300 μL Eluent A or endotoxin-free water to dissolve the plasmid DNA.

[Flow chart]

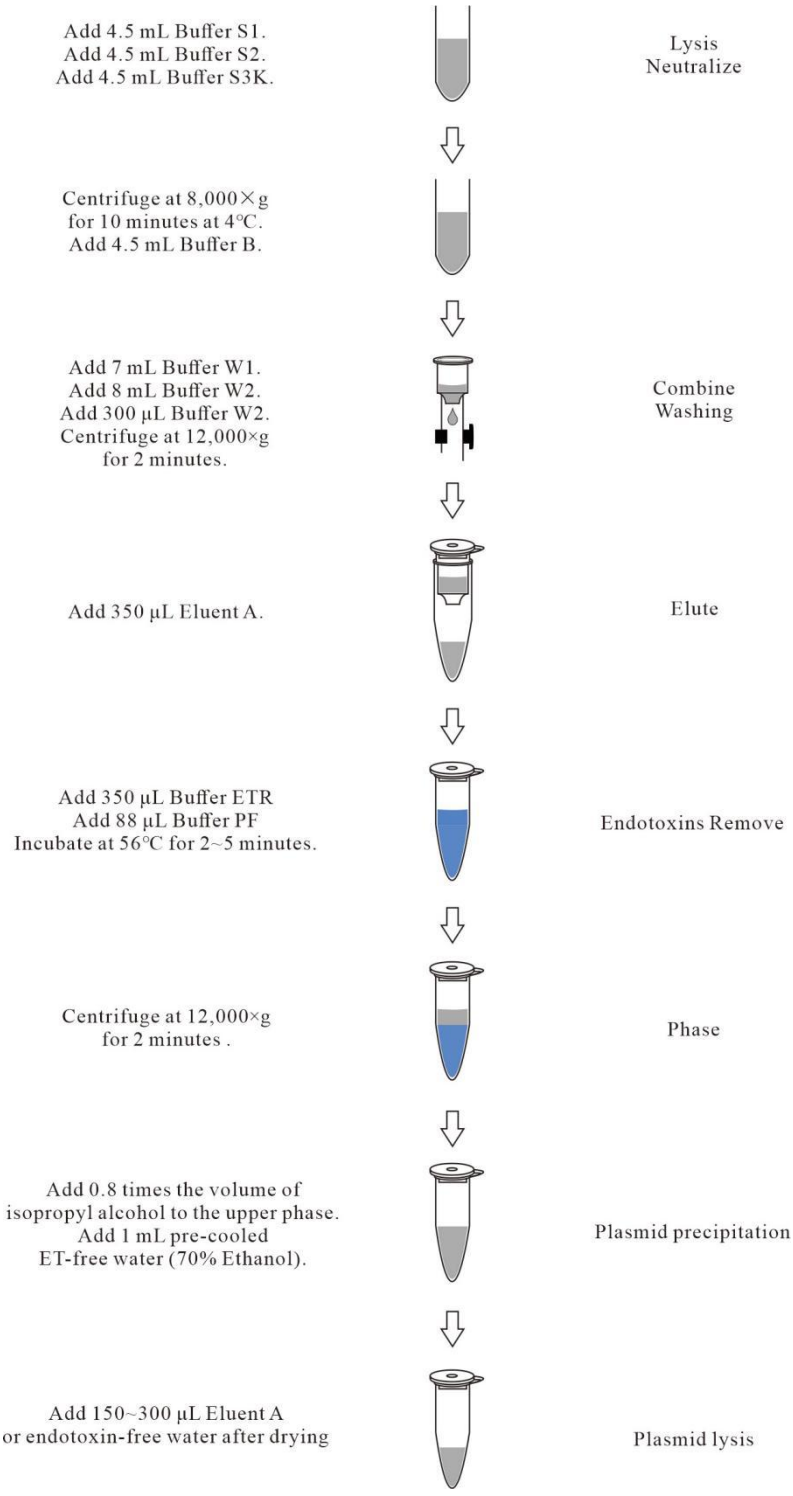

*Figure 1.*The Flow Chart of medium extraction of endotoxin-free plasmid

[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 advice 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

[Instruction Revision Date]

March 07, 2024

[Company Information]

Manufacturer and after-sales service unit Name:

Shenzhen Dakewe Bio-engineering Co., Ltd.

Website:www.dakewe.com

Telephone: (86-755) 86235300

Email: RD@dakewe.com

Production Address: Room 702-703, Building No.1, Shenzhen Biomedicine Innovations Industrial Park, No.14 Jinhui Road, Kengzi Street, Pingshan District, Shenzhen

After-sales service telephone: (86-755) 86235300

Zip Code: 518122