FastPure EndoFree Plasmid Maxi Kit V2

DC212-C1

Vazyme

Instruction for Use

Version 24.2

Vazyme

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

This kit is intended for plasmid DNA extraction from 100 - 300 ml of overnight bacterial cultures. It adopts an optimized alkaline lysis method and features a unique silica membrane adsorption technology and special Buffer ERB and Buffer ERW for specific binding of plasmids and effective removal of endotoxins, proteins, and other impurities. The kit provides high yields of plasmid DNA with minimal endotoxin residue, suitable for use in transfection of various types of cells and common experiments such as enzyme digestion, PCR, sequencing, and ligation.

02/Components

Components	DC212-C1 (10 rxns)
Buffer QB	24 ml
RNase A Solution	3 ml
Buffer P1	120 ml
Buffer P2	120 ml
Buffer P5	60 ml
Buffer ERB	2 × 135 ml
Buffer ERW	120 ml
Buffer PW1	120 ml
Buffer PW2	30 ml
Endotoxin-free Elution Buffer	36 ml
FastPure DNA Maxi Combined Filter	10
FastPure DNA Maxi Column II	10
50 ml Collection Tube	10

Buffer QB: Activate silica membrane and improve yield.
RNase A Solution: Remove RNA.
Buffer P1: Suspend bacteria.
Buffer P2: Lyse bacteria.
Buffer P5: Neutralize lysate.
Buffer ERB: Remove endotoxins and adjust binding conditions.
Buffer ERW: Remove endotoxins and other impurities.
Buffer PW1: Remove proteins and other impurities.
Buffer PW2: Remove residual salt ions.
Endotoxin-free Elution Buffer: Elute plasmid DNA.
FastPure DNA Maxi Combined Filter: Filter impurities.
FastPure DNA Maxi Column II: Adsorb plasmid DNA.
50 ml Collection Tube: Collect flow-through.

03/Storage

Store at 15 ~ 25°C and ship at room temperature.

04/Applications

This kit is intended for large-scale plasmid DNA isolation from 100 - 300 ml of overnight bacterial cultures.

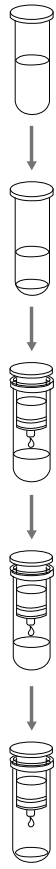
05/Self-prepared Materials

Absolute ethanol, 50 ml sterile centrifuge tubes.

06/Notes

1. The spin columns can be stored at 15 ~ 25°C, or at 2 ~ 8°C for longer storage.
2. Add RNase A Solution to Buffer P1 before use (add all the RNase A Solution provided in the kit) and mix well. After addition of RNase A, the buffer is stable at 2 ~ 8°C for 6 months.
3. Before the first use, add the appropriate volume of absolute ethanol to Buffer PW2 as indicated on the label. Mix well before use.
4. Buffer QB, Buffer P2, Buffer ERB, and Buffer ERW precipitate at low temperatures (<20°C). Check for precipitation before use. Redissolve any precipitate by warming at 37°C, and mix well before use.
5. For low-copy plasmids or large plasmids (>10 kb), use higher volumes of bacterial culture and scale up the volumes of Buffer P1, Buffer P2, and Buffer P5 accordingly. It is recommended to preheat Endotoxin-free Elution Buffer in 65 ~ 70°C water bath and increase incubation and elution times to improve extraction efficiency.
6. The quality and yield of plasmid DNA depend on the plasmid copy number, host bacteria, culture medium, and antibiotics.
7. Do not touch Buffer QB, Buffer P2, Buffer P5, Buffer ERB, or Buffer ERW. Wear gloves when handling these solutions and close the cap tightly immediately after use.
8. When using the filter, remove the plunger slowly from the syringe to avoid disturbing the filter membrane.
9. All centrifuge steps are carried out at room temperature (15 ~ 25°C).

07/Mechanism & Workflow



Resuspend
◇ Add 10 ml of Buffer P1

Lyse
◇ Add 10 ml of Buffer P2

Neutralize
◇ Add 5 ml of Buffer P5

Remove endotoxins
◇ Add 1 × volume of Buffer ERB

Bind
◇ Transfer all the mixture to the spin column

Wash
◇ Add 10 ml of Buffer ERW
◇ Add 10 ml of Buffer PW1
◇ Add 12 ml of Buffer PW2

Elute
◇ Add 1 - 3 ml of Endotoxin-free Elution Buffer

08/Experiment Process

1. Equilibration: Place a FastPure DNA Maxi Column II in a 50 ml Collection Tube. Add 2 ml of Buffer QB to the spin column and centrifuge at 8,200 rpm (8,000× g) for 2 min. Discard the flow-through.
   ▲ Equilibration is required for spin columns stored at high temperatures.
   ▲ Use equilibrated columns as soon as possible to ensure maximum yield.
   ▲ Centrifuge speed can be reduced to 4,600 × g (6,000 rpm). Increase centrifuge duration as appropriate.

2. Centrifuge 100 - 300 ml of overnight culture at 8,200 rpm (8,000 × g) for 3 min. Remove as much supernatant as possible.
   ▲ When using 2 × YT, TB, and other enriched growth medium, it is recommended to process 150 ml of overnight culture due to the high cell density. If more than 150 ml of culture is used, scale up the volumes of Buffer P1, Buffer P2, and Buffer P5 accordingly.

3. Add 10 ml of Buffer P1 (ensure RNase A Solution has been added) and vortex until the cells are completely resuspended. The suspension should be homogenous and no cell pellets or clumps should remain.
   ▲ Immediately proceed to the next step after cell resuspension to avoid cell clumping and insufficient lysis.

4. Immediately add 10 ml of Buffer P2 and mix gently by inverting the tube 10 - 15 times. Incubate at room temperature for 5 min.
   ▲ Mix the solution gently. Do not agitate vigorously to avoid contamination of genomic DNA. The solution should be clear and viscous. Do not take more than 5 min to avoid denaturation of plasmid DNA. For cell densities over 220 OD (based on the measured OD600), increase the number of inversions to lyse the cells completely. If the solution does not become clear, lysis may have been incomplete due to an excess amount of cells. In this case, a smaller volume of bacterial culture should be used.
   ▲ For ultra-low temperature freeze-thaw samples, add Buffer P2 and wait for the solution to become clear or incubate at room temperature for 3 min, then proceed with the steps.

5. Add 5 ml of Buffer P5 and immediately mix by inverting the tube gently 8 - 12 times to neutralize Buffer P2. The solution should become cloudy with a white, evenly dispersed flocculent precipitate. Centrifuge at 8,200 rpm (8,000 × g) for 10 min. Transfer the supernatant to a new sterile centrifuge tube.
   ▲ For cell densities over 220 OD, increase the number of inversions to fully neutralize the solution. Mix gently and thoroughly immediately after adding Buffer P5 to avoid localized precipitation. The supernatant after centrifugation should be clear; small white floccules at the surface do not interfere with subsequent steps.
   ▲ The centrifugal conditions are compatible to 6,000 rpm (4,600 × g), and the centrifugal time needs to be extended to 30 min when using low centrifugal force to fully precipitate and remove the flocculent; If the input amount of bacteria exceeds 200 ml, it is recommended to extend the centrifugation time to 20 min.

6. (Optional) Carefully transfer the supernatant from last step to a FastPure DNA Maxi Combined Filter (do not add large amounts of precipitate as this may clog the filter). Slowly push the plunger to pass the solution through the filter and collect the flow-through in a new 50 ml sterile centrifuge tube (not provided).

7. Add an equal volume of Buffer ERB to the obtained supernatant. Mix by inverting the tube 8 times.
   ▲ Buffer ERB foams easily. Do not shake vigorously after adding it to the solution.

8. Transfer the mixture from Step 7 to a FastPure DNA Maxi Column II. Centrifuge at 8,200 rpm (8,000 × g) for 1 - 2 min, and discard the flow-through.
   ▲ The maximum reservoir capacity of the spin column is 12 ml. If the volume of the mixture exceeds this capacity, load it onto the column in multiple applications. For fixed-angle rotors with large angles, reduce the volume loaded into the column to avoid spills.

9. Repeat Step 8 until all of the mixture is loaded.

10. Add 10 ml of Buffer ERW to the spin column and centrifuge at 8,200 rpm (8,000 × g) for 2 min. Discard the flow-through.
11. Add 10 ml of Buffer PW1 to the spin column and centrifuge at 8,200 rpm (8,000 × g) for 2 min. Discard the flow-through.
12. Add 12 ml of Buffer PW2 (ensure that absolute ethanol has been added) and centrifuge at 8,200 rpm (8,000 × g) for 2 min. Discard the flow-through.
13. Place the spin column back into the Collection Tube and centrifuge at 8,200 rpm (8,000 × g) for 5 min.
14. Place the spin column in a new 50 ml sterile centrifuge tube (supplied in the kit) and air dry at room temperature for a few minutes. Add 1 - 3 ml of Endotoxin-free Elution Buffer to the center of the spin column membrane. Incubate at room temperature for 3 - 5 min and centrifuge at 8,200 rpm (8,000 × g) for 3 min. Discard the spin column.
    ▲ Residual ethanol in the spin column will interfere with downstream experiments.
    ▲ Do not use an elution volume lower than 1 ml as this may decrease elution efficiency.
    ▲ When eluting with ddH_2O, ensure the pH is within 7.0 - 8.5, as lower pH will decrease elution efficiency.
    ▲ To improve elution efficiency, preheat the Elution Buffer at 65°C or perform an additional elution step.
15. Store the extracted plasmid DNA at -30 ~ -15°C.

Optional Steps (for higher plasmid concentrations, perform the following steps):

16. For each 1 ml of Endotoxin-free Elution Buffer, add 0.1 volumes of 3 M NaAc (pH 5.2) and 2 volumes of pre-chilled absolute ethanol. Mix well and incubate at -20°C for 10 - 20 min. Centrifuge at 12,560 rpm (14,000 × g) for 20 min at 4°C. Carefully discard the supernatant.
17. Add 1 ml of 70% ethanol to wash the pellet. Centrifuge at 12,560 rpm (14,000 × g) for 3 min at 4°C. Carefully discard the ethanol.
18. Air dry the pellet for 5 - 10 min to aviod ethanol residue. Resuspend the pellet in an appropriate volume of Endotoxin-free Elution Buffer based on the application.

09/FAQ & Troubleshooting

Problem	Cause	Solution
Low yield	1. Low copy plasmid	Differences in copy number can cause significant variations in plasmid yield. For low-copy plasmids (SuperCos, pWE15, pBR322, pACYC and its derivatives, pSC101 and its derivatives) or plasmids with large inserts (>10 kb), use higher volumes of bacterial culture and scale up the volumes of Buffer P1, Buffer P2, and Buffer P5 accordingly; preheat the Elution Buffer to 65 ~ 70°C and increase incubation and elution time to improve extraction efficiency.
	2. No plasmids in cells	Ensure the correct antibiotic and working concentration are used for screening. The stability of plasmids varies in different host strains; avoid plasmid loss and damage due to repeated subculturing.
	3. Incomplete resuspension of cells	Cell resuspension is crucial to plasmid yield. Ensure that the cell pellet is completely resuspended and no cell clumps are visible. Clumped cells cannot be lysed and will reduce the yield.
	4. Reagent preparation errors	Ensure all RNase A Solution has been added to Buffer P1 before use. Buffer P2 will precipitate at low temperatures or after prolonged storage. Before use, redissolve any precipitate by warming or place the buffer in a 37°C incubator until the solution becomes clear. Ensure the correct volume of absolute ethanol has been added to Buffer PW2.
	5. Unequilibrated spin column	Prolonged exposure of the spin columns to poor storage conditions will reduce extraction efficiency. Before use, treat the spin column with Equilibration Buffer QB to activate the silica membrane and improve yield.
Low purity	1. Residual gDNA	After adding Buffer P2, mix by gentle inversion of the tube; when processing multiple samples, ensure that the lysis time does not exceed 5 min.
	2. Residual salt ions	Ensure that the column is washed once with Buffer PW1 and once with Buffer PW2; add the wash buffer along the wall of the column to reduce residual ions.
	3. Residual RNA	After addition of RNase A to Buffer P1, prolonged exposure to room temperature may result in loss of enzyme activity. Put the buffer to 2 ~ 8°C promptly after use. Excessive amounts of cells may lead to insufficient digestion of bacterial RNA by RNase A in Buffer P1. In this case, reduce the bacterial culture volume.

High residual endotoxin	1. Incomplete mixing after addition of Buffer P5	Mix thoroughly after adding Buffer P5 by inverting up to 20 times. The supernatant should be clear after centrifugation.
	2. Buffer ERB precipitates	Buffer ERB may precipitate at low temperatures. Warm to 37°C until fully dissolved and mix well before use.
	3. Incomplete mixing after addition of Buffer ERB	Mix thoroughly after adding Buffer ERB by inverting up to 15 times.

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