FastPure EndoFree Plasmid Mini Kit

DC203

Vazyme

Instruction for Use

Version 24.2

Contents

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

01/Product Description

This kit is intended for plasmid DNA extraction from 1 - 5 ml of overnight bacterial cultures. It adopts an optimized alkaline lysis method and features a unique silica gel 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 whole extraction process takes only 25 min. The extracted plasmid DNA contains minimal endotoxin residue (≤0.1 EU/μg) and can be used for the transfection of various types of cells and common experiments such as enzyme digestion, PCR, sequencing, and ligation.

02/Components

Components	DC203-01 (50 rxns)
RNase A Solution	375 μl
Buffer QB	5 ml
Buffer P1	15 ml
Buffer P2	15 ml
Buffer P5	8 ml
Buffer ERB	35 ml
Buffer ERW	30 ml
Buffer PW1	30 ml
Buffer PW2	10 ml
Endotoxin-free Elution Buffer	10 ml
FastPure DNA Mini Columns II	50
Collection Tubes 2 ml	50

RNase A Solution: Remove RNA.
Buffer QB: Equilibrate spin columns.
Buffer P1: Resuspend bacteria.
Buffer P2: Lyse bacteria.
Buffer P5: Neutralize the solution.
Buffer ERB: Remove endotoxins and provide an environment for binding.
Buffer ERW: Remove endotoxins and other impurities.
Buffer PW1: Remove impurities such as protein.
Buffer PW2: Remove residual salt ions.
Endotoxin-free Elution Buffer: Elute plasmid DNA.
FastPure DNA Mini Columns II: Adsorb plasmid DNA.
Collection Tubes 2 ml: Collect filtrate.

03/Storage

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

04/Applications

1 - 5 ml of overnight bacterial culture.

For low-copy plasmids, it is recommended to increase the bacterial culture volume to 5 - 10 ml and scale up the volumes of Buffer P1, Buffer P2, and Buffer P5.

05/Self-prepared Materials

Absolute ethanol, 1.5 ml and 2 ml sterile centrifuge tubes, etc.

06/Notes

1. The spin columns can be stored at 15 ~ 25°C but should be stored at 2 ~ 8°C for long-term preservation.
2. Before the first use, add all the RNase A Solution supplied in the kit to Buffer P1 and mix well. Store the mixture at 2 ~ 8°C for up to 6 months.
3. Before the first use, add an appropriate volume (40 ml) of absolute ethanol to Buffer PW2 according to the label.
4. Precipitates may appear in Buffer QB, Buffer P2, Buffer ERB, and Buffer ERW at low temperatures. Check for crystal precipitation before use. Dissolve any precipitate completely by warming the buffers at 37°C, and mix the buffers well before use.
5. When preparing low-copy plasmids, increase the volume of bacterial culture as appropriate and scale up the volumes of Buffer P1, Buffer P2, and Buffer P5.
6. Different copy numbers of plasmid vectors will lead to significant fluctuations in DNA yield. High-copy vectors yield approximately 10 - 50 μg of DNA per 1 - 5 ml of bacterial culture, while low-copy vectors yield approximately 2 - 5 μg of DNA per 5 - 10 ml of culture.
7. Avoid direct contact with Buffer QB, Buffer P2, Buffer P5, Buffer ERB, and Buffer ERW. Wear latex gloves when handling the buffers and tighten the bottle caps immediately after use.
8. Perform all steps at room temperature.
9. The force acting on a centrifuge depends on the radius of a particular rotor. If an experiment is performed the same way every time, RCF (× g) must stay constant.

07/Mechanism & Workflow

Resuspend — Add 250 µl of Buffer P1

Lyse — Add 250 µl of Buffer P2

Neutralize — Add 125 µl of Buffer P5

Centrifuge to collect the supernatant

Remove endotoxins — Add Buffer ERB equal to the supernatant volume

Bind — Transfer all mixture to the spin column

Rinse — Add 500 µl of Buffer ERW

Add 500 µl of Buffer PW1

Add 700 µl of Buffer PW2

Elute — Add 30 - 100 µl of Endotoxin-free Elution Buffer

08/Experiment Process

1. Column equilibration: Place FastPure DNA Mini Columns II into a Collection Tube 2 ml. Add 100 µl of Buffer QB to FastPure DNA Mini Columns II, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the filtrate (Treat the spin column on the day of use).
2. Centrifuge 1 - 5 ml of overnight culture at 12,000 rpm (13,400 × g) for 1 min to pellet the bacterial cells, and remove the supernatant as completely as possible.
3. Add 250 µl of Buffer P1 (make sure that RNase A Solution has been added) and vortex until the cells are completely resuspended.
4. Add 250 µl of Buffer P2 and mix well by gently inverting the tube 8 - 10 times. Incubate at room temperature for 3 min.

   ▲ Mix gently. Do not shake vigorously to avoid genomic DNA contamination. The mixture should become clear and viscous. Do not incubate for more than 5 min to avoid plasmid damage. If the solution fails to become clear, too many bacterial cells may have been used, leading to incomplete lysis. Reduce the cell input.

5. Add 125 µl of Buffer P5. Immediately invert the tube gently 12 - 15 times to fully neutralize Buffer P2. White flocculent precipitates should form. Centrifuge the system at 12,000 rpm (13,400 × g) for 10 min.
   ▲ Mix immediately after adding Buffer P5 to avoid localized precipitation. The supernatant should be clear after centrifugation. Small white precipitates in the supernatant will not interfere with subsequent steps.
6. Transfer the supernatant (about 600 µl) to a new 1.5 ml sterile centrifuge tube (not provided). Add an equal volume of Buffer ERB and mix by inverting the tube 10 times.
   ▲ Buffer ERB foams easily. Avoid vigorous vortex after adding the buffer.
7. Transfer the mixture from Step 6 to FastPure DNA Mini Columns II (fitted in a Collection Tubes 2 ml), and centrifuge at 12,000 rpm (13,400 × g) for 1 min. Discard the filtrate.
   ▲ The capacity of the spin column is 750 µl. If the volume of the mixture exceeds this limit, load the mixture onto the column in multiple times.
8. Repeat Step 7 until all of the mixture has been applied.
9. Add 500 µl of Buffer ERW to the spin column, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the filtrate.
10. Add 500 µl of Buffer PW1 to the spin column, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the filtrate.
11. Add 700 µl of Buffer PW2 (make sure that absolute ethanol has been added) to the spin column, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the filtrate.
12. Place the FastPure DNA Mini Columns II back into the Collection Tubes 2 ml, and centrifuge at 12,000 rpm (13,400 × g) for 2 min.
13. Place the FastPure DNA Mini Columns II in a new 1.5 ml sterile centrifuge tube (not provided). Add 30 - 100 µl of Endotoxin-free Elution Buffer to the center of the FastPure DNA Mini Columns II membrane. Incubate at room temperature for 1 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min and discard the FastPure DNA Mini Columns II.
    ▲ We recommend an elution volume of no less than 30 µl, as lower volumes reduce elution efficiency.
    ▲ If ddH_2O is used for elution, make sure that the pH is within the range of 7.0 - 8.5, as a pH below 7.0 reduces elution efficiency.
    ▲ To make the elution more efficient, pre-heat Elution Buffer at 65°C.
14. Store the extracted plasmid DNA at -30 ~ -15°C.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Low plasmid DNA yield	1. Low-copy plasmids	Different copy numbers of plasmid vectors will lead to significant fluctuations in DNA yield. For low-copy plasmids, increase the sample volume to 5 - 10 ml of overnight culture and scale up the volumes of Buffer P1, Buffer P2, and Buffer P5; pre-heat Elution Buffer at 65°C to enhance extraction efficiency. The other steps remain the same.
	2. Large plasmids (>10 kb)	Increase the culture volume to 5 - 10 ml and scale up the volumes of Buffer P1, Buffer P2, and Buffer P5; pre-heat Elution Buffer at 65°C to enhance extraction efficiency. The other steps remain the same.
	3. Host strain variation	Plasmid yield is also affected by host strains. We recommend using *endA- E. coli* strains, such as DH5α, TOP10, and XL10.
	4. Incomplete cell lysis	Make sure that bacterial cells are completely resuspended in Buffer P1. Cell clumps will lead to lower yields due to incomplete lysis.
	5. Precipitation in Buffer P2	Precipitate may appear in Buffer P2 at low temperatures. Warm at 37°C until the precipitate is completely dissolved, and mix the buffer well before use.
	6. Improper bacteria strain storage	Plasmid loss occurred during the preservation of the bacteria strain. It is advisable to revive bacteria by streaking or spreading onto a special plate before further culturing to ensure the yield.
	7. Culture overgrown	Limit bacterial growth within 16 h (preferably within 12 - 14 h).
	8. Long-term exposure of spin columns to adverse environmental conditions (e.g., high temperatures)	Perform column equilibration (08/Experiment Process/Step 1).
Genomic DNA contamination	1. Improper lysis	Mix the sample by gentle inversion after adding Buffer P2; when processing multiple samples, the lysis time should not exceed 5 min.
Low purity	1. Residual salt ions	Ensure that the column is rinsed once each with Buffer PW1 and Buffer PW2. We recommend adding the wash buffers along the wall of the spin column, or capping the column and inverting 2 - 3 times after buffer addition to facilitate salt ion removal.
	2. Residual ethanol	After centrifuging the empty column, allow it to stand uncapped at room temperature for 2 min to fully remove residual ethanol.
High levels of residual endotoxin	1. Insufficient mixing after addition of Buffer P5	Mix the solution thoroughly after adding Buffer P5. Invert the tube up to 20 times to ensure that a clear supernatant is obtained after centrifugation.
	2. Precipitation in Buffer ERB	Precipitate may appear in Buffer ERB at low temperatures. Warm at 37°C until the precipitate is completely dissolved, and mix the buffer well before use.
	3. Insufficient mixing after addition of Buffer ERB	Mix the solution thoroughly after adding Buffer ERB. Invert the tube up to 15 times.
RNA residue	1.Decreased RNase A activity	The activity of RNase A in Buffer P1 may decrease if the buffer is left at room temperature for a long time. Return the buffer to 2 ~ 8°C promptly after use.
	2.Too much bacterial culture	The RNase A in Buffer P1 cannot digest all RNA in the bacterial cells. Reduce the bacterial culture volume as appropriate.

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