FastPure EndoFree Plasmid Midi Kit

DC205

Vazyme

Instruction for Use

Version 24.2

Contents

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

01/Product Description

This kit is applicable for plasmid DNA extraction from bacterial culture in a volume of 15 - 50 ml. It uses an optimized alkaline lysis method to lyse cells and utilizes a unique silica gel membrane adsorption technique along with special Buffer ERB and Buffer ERW solutions to specifically bind plasmids and effectively remove impurities such as endotoxins, proteins, and genomic DNA. Additionally, the unique P2 solution with indicator can indicate the completeness of lysis and neutralization through color changes, ensuring extraction quality and enabling visual operation. The extracted plasmid DNA has extremely low residual endotoxin levels, making it suitable for various cell transfection and routine operations, including restriction endonuclease digestion, PCR, sequencing, ligation, and other experiments.

02/Components

Components	DC205-01 (10 rxns)
RNase A Solution	800 µl
Buffer QB	11 ml
Buffer P1	28 ml
Buffer P2	28 ml
Buffer P5	14 ml
Buffer ERB	66 ml
Buffer ERW	22 ml
Buffer PW1	33 ml
Buffer PW2	9 ml
Endotoxin-free Elution Buffer	11 ml
FastPure DNA Midi Columns	10
Collection Tubes 15 ml	10

03/Storage

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

04/Applications

15 - 50 ml of overnight bacterial culture.

05/Self-prepared Materials

Absolute ethanol, 15 ml sterile centrifuge tube, etc.

06/Notes

1. Store spin column at 15 ~ 25°C or 2 ~ 8°C for long-term storage.
2. Before the first use, add the RNase A Solution (add all the RNase A Solution provided 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 the corresponding volume of absolute ethanol as indicated on the Buffer PW2 bottle 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. The yield and quality of plasmid DNA depend on factors such as plasmid copy number, host strain, inoculation volume, culture medium type, and antibiotics.
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. 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.
9. Perform all steps at room temperature.

07/Mechanism & Workflow

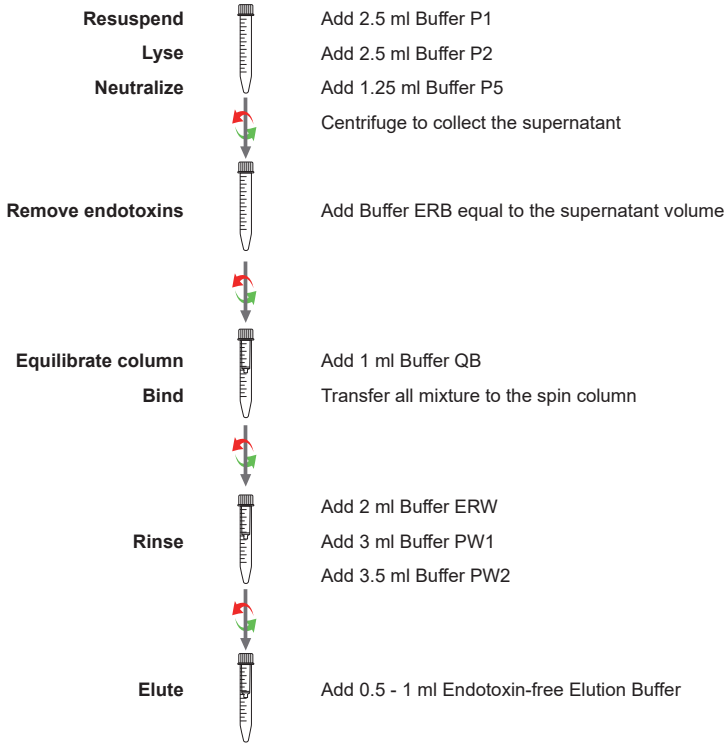

Fig 1. Workflow of FastPure EndoFree Plasmid Midi Kit

08/Experiment Process

08-1/ Procedure

1. Centrifuge 15 - 50 ml overnight bacterial culture at 5,000 rpm (4,600 × g) for 5 min to pellet the bacterial cells, and remove the supernatant as completely as possible.
2. Add 2.5 ml Buffer P1 (please check if RNase A Solution has been added), and vortex until the cells are completely resuspended.
   ▲ After complete resuspension of the bacterial cells, subsequent operations should be carried out immediately to prevent cell aggregation and incomplete lysis.
3. Immediately add 2.5 ml Buffer P2, gently mix by inversion 10 - 15 times, and leave at room temperature for 5 min.
   ▲ This step should be gently mixed, avoiding vigorous shaking to prevent contamination of genomic DNA. At this time, the solution should gradually change from a turbid and viscous blue color to a clear and sticky blue color, indicating complete lysis. The duration should not exceed 5 min to avoid destruction of the plasmids. If the solution is not clear, there may be too many bacteria resulting in incomplete lysis, so the amount of bacteria should be reduced appropriately.

4. Add 1.25 ml Buffer P5, gently invert 10 - 15 times, until the blue color completely disappears. A white flocculent precipitate should appear at this time. Centrifuge at 5,000 rpm (4,600 × g) for 10 min.
   ▲ After adding Buffer P5, gently mix by inversion immediately to avoid localized precipitation. At this time, the solution will change from blue to colorless, indicating complete neutralization. After centrifugation, the upper clear layer should be in a clarified state. If there are small white precipitates floating on the surface of the supernatant, it does not affect subsequent operations.

5. Transfer the upper clear layer (approximately 6 ml) to a new 15 ml sterile centrifuge tube (self-prepared materials), add an equal volume of Buffer ERB, and mix by inversion 10 - 15 times.
   ▲ After adding Buffer ERB reagent, mix by inversion to avoid vigorous shaking.

6. **Equilibrate column:** Add 1 ml Buffer QB to the FastPure DNA Midi Columns, centrifuge at 5,000 rpm (4,600 × g) for 1 min, discard the filtrate, and set aside.
   ▲ The equilibration buffer can activate the ability of the silica gel membrane. Please use the spin column processed on the same day.

7. Transfer the mixture in step 5 to the spin column, centrifuge at 5,000 rpm (4,600 × g) for 2 min, discard the filtrate.
   ▲ The maximum volume of the spin column is 4 ml. If the mixture exceeds this volume, please load it onto the column multiple times.

8. Repeat step 7 until the entire mixture is loaded onto the column.
   ▲ When adding liquid, please add it along the tube wall. Otherwise, it may cause damage to the surface of the adsorption membrane, but it does not affect the performance.

9. Add 2 ml Buffer ERW to the spin column, centrifuge at 5,000 rpm (4,600 × g) for 2 min, discard the filtrate.

10. Add 3 ml Buffer PW1 to spin column, centrifuge at 5,000 rpm (4,600 × g) for 2 min, discard the filtrate.

11. Add 3.5 ml Buffer PW2 (please check if absolute ethanol has been added) to the spin column, centrifuge at 5,000 rpm (4,600 × g) for 2 min. Discard the filtrate.

12. Place the empty spin column back into the collection tube, centrifuge at 5,000 rpm (4,600 × g) for 5 min.

13. Transfer the spin column to a new sterile 15 ml centrifuge tube (self-prepared materials), air dry at room temperature for 3 - 5 min, add 0.5 - 1 ml of Endotoxin-free Elution Buffer to the center of the spin column membrane. Let it stand at room temperature for 3 min, centrifuge at 5,000 rpm (4,600 × g) for 5 min, discard the spin column.
   ▲ The suggested elution volume should not be less than 0.5 ml. A smaller volume will reduce the elution efficiency.
   ▲ If using ddH_2O elution, ensure that the pH of ddH_2O is within the range of 7.0 - 8.5. A pH lower than 7.0 will decrease elution efficiency.
   ▲ To increase the plasmid yield, transfer the eluate to the spin column for secondary elution.

14. The extracted plasmid DNA is stored at -30 ~ -15°C.

08-2/Optional Steps

If higher concentration of plasmid is needed, the following concentration operation can be carried out:

1. Add an equal volume of isopropanol and 0.1 times the volume of Buffer P5 to every 1 ml of eluted product, mix well, and let it stand at room temperature for 5 min.
2. Centrifuge at room temperature at 12,000 rpm (13,400 × g) for 10 min, carefully discard the supernatant.
3. Add 1 ml 70% ethanol to wash the precipitate, centrifuge at room temperature at 12,000 rpm (13,400 × g) for 3 min, carefully discard ethanol.
4. Repeat operation step 3.
5. Air dry precipitate for about 5 - 10 min, dissolve the precipitate with an appropriate volume of Endotoxin-free Elution Buffer as needed.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Low DNA yield	1. Low-copy plasmids	Differences in copy number will result in significant fluctuations in plasmid yield. For low-copy plasmids, such as pET, pWE15, SuperCos, pBR322, pACYC and derivatives, pSC101 and derivatives, the amount of bacteria should be increased. 100 ml of bacterial cultures should be used and the volumes of Buffer P1, Buffer P2, and Buffer P5 should be increased proportionally. Elution should be preheated in a 65°C water bath to increase extraction efficiency. The other steps remain the same.
	2. Large plasmids (>10 kb)	Elution should be preheated in a 65°C water bath to increase 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. 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.
	5. 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.
	6. Culture overgrown	Limit bacterial growth within 16 h (preferably within 12 - 14 h).
	7. Long-term exposure of spin columns to adverse environmental conditions (e.g., high temperatures)	Perform 08-1/Procedure/Step 6
Genomic DNA contamination	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 5 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 cultures	Due to the excessive number of bacteria (>100 ml), RNase A in Buffer P1 is insufficient to digest RNA in bacteria. It is recommended to reduce bacterial cultures volume.
Clogged adsorption columns	Centrifuge centrifugal force is too small	The same speed of different centrifuges corresponds to different centrifugal forces. The centrifugal force should be at 2,500 × g ~ 8,000 × g.

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