8 min FastPure Plasmid Mini Kit

DC221

Vazyme

Instruction for Use

Version 25.1

Contents

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

01/Product Description

This kit is suitable for extracting 1 - 4 ml of overnight cultured bacterial culture, utilising a optimized alkaline lysis method to lyse cells, which improves the efficiency of nucleic acid release. It employs a unique silica matrix membrane adsorption technology to effectively bind plasmids and remove proteins, polysaccharides, and other impurities, resulting in high-purity plasmid DNA.

The addition of a unique P2 solution with an indicator provides visual feedback through color changes, signaling whether lysis and neutralization are adequate, thereby ensuring extraction quality and facilitating visual operation. The yield of extracted plasmid DNA is high, requiring only 8 min, and the purified plasmid DNA can be directly used for restriction endonuclease digestion, PCR, sequencing, ligation, conversion, transfection of certain conventional cell lines, and other biological experiments.

02/Components

	Components	DC221-01 (150 rxns)	DC221-02 (300 rxns)
■	RNase A Solution	112.5 µl	225 µl
	Buffer P1	22.5 ml	45 ml
	Buffer P2	22.5 ml	45 ml
	Buffer NP3	52.5 ml	105 ml
	Buffer PW	24 ml	48 ml
	Elution Buffer	15 ml	30 ml
	FastPure DNA Mini Columns IV	150	2 × 150
	2 ml Collection Tubes	150	2 × 150

RNase A Solution: Remove RNA;
Buffer P1: Suspend bacteria;
Buffer P2: Lyse bacteria;
Buffer NP3: Neutralization;
Buffer PW: Remove protein, ironic salts and other impurities residues;
Elution Buffer: Elute plasmid;
FastPure DNA Mini Columns IV: Adsorb plasmid;
2 ml Collection Tubes: Collect filtrate.

03/Storage

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

04/Applications

It is applicable to 1 - 4 ml overnight cultured bacterial culture.

05/Self-prepared Materials

Absolute ethanol, 2 ml sterile centrifuge tube and 1.5 ml sterile centrifuge tube.

06/Notes

1. Store spin column at 15 ~ 25°C for short time. If ambient temperatures often exceed 25°C, we suggest storing at 2 ~ 8°C.
2. Before using Buffer P1, please add RNase A Solution (add all the RNase A Solution provided in the kit). Mix well and store at 2 ~ 8°C. The mixed RNase A can be stored up to 6 months.
3. Before the first use, add the corresponding volume of absolute ethanol according to the label on the Buffer PW bottle. Mix well for use.
4. Buffer P2, Buffer NP3 may produce white precipitate when stored at low temperatures (<20°C). In this case, leave the buffers at room temperature for a while before use, or incubate in a 37°C water bath if necessary until the precipitate is completely dissolved, and mix well before use.
5. When handling low-copy plasmids or large fragment plasmids (>10 kb), the bacterial culture input can be increased, and the usage of Buffer P1, Buffer P2, and Buffer NP3 can be increased proportionally. It is recommended to preheat the Eluion Buffer in a 65°C water bath and appropriately extend the adsorption and elution time to improve the yield.
6. The yield and quality of plasmid DNA are related to factors such as plasmid copy number, host bacteria, culture medium type, antibiotics, etc.
7. Note that direct contact with Buffer P2, Buffer NP3 should be avoided. Latex gloves should be worn when handling these reagents, and the caps should be tightly closed immediately after use to prevent any possible reagent degradation, which could reduce the extraction efficiency. Avoid direct contact with Buffer P2, Buffer NP3. Wear gloves when handling the buffers and tighten the bottle caps immediately after use.
8. All centrifugation steps are carried out at room temperature (15 ~ 25°C).

07/Mechanism & Workflow

Resuspend: Add 150 µl Buffer P1, vortex, and resuspend bacterial cells.

Lyse visualization: Add 150 µl Buffer P2, and invert gently 15 - 18 times.

Neutralize: Add 350 µl Buffer NP3, and invert rapidly up and down immediately 15 - 25 times until the blue color disappears. Flocculent precipitate dispersed uniformly.

Centrifuge at 12,000 rpm (13,400 × g) for 2 min. Collect the supernatant.

Binding: Transfer the entire mixture to spin column.

Washing: Add 700 µl Buffer PW, 12,000 rpm (13,400 × g) centrifuge for 30 sec.

Centrifuge empty column at 12,000 rpm (13,400 × g) for 1 min.

Elution: Add 30 - 80 µl Elution Buffer. Centrifuge at 12,000 rpm (13,400 × g) for 30 sec - 1 min.

Fig 1. 8 min FastPure Plasmid Mini Kit extraction procedure

08/Experiment Process

1. Take 1 - 4 ml (2 - 8 OD) overnight cultured bacterial cultures, centrifuge at 12,000 rpm (13,400 × g) for 1 min to collect the bacterial cells and remove as much supernatant as possible without disturbing the pellet.
   ▲ Any excess residual cell culture medium will reduce lysis buffer efficiency; discard as much as possible.
2. Add 150 µl Buffer P1 (please check if RNase A Solution has been added first). Use a pipette or vortex mixer to mix until the bacteria are completely resuspended. The resuspended solution should appear as a homogeneous dispersed state with no obvious bacterial particles or clumps.
   ▲ Subsequent operations should be performed immediately after the bacterial cells are completely resuspended. Otherwise, the bacterial cells may clump together and are inadequately lysed, which negatively impacts extraction performance.
   ▲ When processing low-copy plasmids or large fragment plasmids (>10 kb), the bacterial culture input volume can be increased by one fold, and the usage of Buffer P1, Buffer P2, and Buffer NP3 should be increased proportionally.

3. Immediately add 150 µl Buffer P2, gently mix by inversion 15 - 18 times. Bacterial culture should appear as a clear and blue liquid.
   ▲ This step should be done gently, avoiding vigorous shaking to reduce the risk of genomic DNA contamination. Bacterial culture should become clear and viscous. This step should not exceed 3 min to avoid damaging the plasmid.
   ▲ Note to ensure all bacterial cells are mixed with lysis buffer!
4. Add 350 µl Buffer NP3, immediately invert gently 15 - 25 times until the blue colour completely disappears. At this point, a white uniformly dispersed flocculent precipitate should appear. Centrifuge at 12,000 rpm (13,400 × g) for 2 min.
   ▲ The solution changes from blue to colorless, indicating complete neutralization. Avoid the formation of large amounts of aggregated precipitates, otherwise it will affect the extraction yield and pipetting operation after centrifugation. After centrifugation, the supernatant should be clear. If there are tiny white precipitates floating on the surface of the supernatant, it does not affect subsequent operations.
   ▲ Buffer P2, Buffer NP3 may produce white precipitate when stored at low temperatures. In this case, leave the buffers at room temperature for a while before use, or incubate in a 37°C water bath if necessary until the precipitate is completely dissolved, and mix well before use.
   ▲ Optional: Add 100 µl 3 M NaOH to the adsorption column, let it stand for 30 sec. Centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate. If columns are stored at room temperature for a long time, alkaline treatment is recommended to improve binding efficiency.
5. Carefully transfer the supernatant after centrifugation to FastPure DNA Mini Columns IV spin column (place in 2 ml Collection Tubes). Centrifuge at 12,000 rpm (13,400 × g) for 30 sec, and discard the filtrate.
6. Add 700 µl Buffer PW (please check if absolute ethanol has been added) to the spin column. Centrifuge at 12,000 rpm (13,400 × g) for 30 sec, and discard the filtrate.
   ▲ If it is a high-protein expression bacterial strain (such as JM109, Stbl3, etc.), one more time Buffer PW can be added to wash thoroughly to remove impurities.
7. Place the spin column into the collection tube, centrifuge at 12,000 rpm (13,400 × g) for 1 min, and discard the collection tube.
   ▲ Open the lid and air dry to completely remove any ethanol residue. Ethanol residue can affect the downstream protocols.
8. Place the spin column in a new 1.5 ml sterile centrifuge tube (self-prepared) and add 30 - 80 µl Elution Buffer directly on the center of the spin column membrane. Centrifuge at 12,000 rpm (13,400 × g) for 30 sec - 1 min, and discard spin column.
   ▲ Elution volume is recommended to be no less than 30 µl. A smaller volume will reduce elution efficiency.
   ▲ If ddH_2O is used for elution, it should be ensured that the pH of ddH_2O is in the range of 7.0 - 8.5. A pH lower than 7.0 will reduce the elution efficiency.
   ▲ Preheat or re-elute the eluate at 65°C, which can improve the plasmid elution efficiency.
9. The extracted plasmid DNA can be stored at -30 ~ -15°C.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Low yields	1. Plasmid with low copy number	The yield of plasmids can fluctuate significantly due to differences in copy numbers. When processing low-copy plasmids (such as SuperCos, pWE15, pBR322, pACYC and their derivatives, pSC101 and its derivatives) or large fragments (>10 kb), it is recommended to appropriately increase the bacterial culture input and proportionally increase the amounts of Buffer P1, Buffer P2, and Buffer NP3; pre-heat the elution buffer to 65 ~ 70°C, and extend the adsorption and elution times appropriately to improve recovery rates.
	2. No plasmids in the organism	To ensure effective screening, select the appropriate antibiotics and working concentrations, as plasmid stability varies among different host bacteria. This helps prevent plasmid loss and potential damage from multiple iterative transfers. During the preservation of glycerophosphate bacterial strains, plasmid loss may occur. To stabilize yield, it is advisable to streak or plate the strain before initiating culture.
	3. Host strain differences	Plasmids yield can also be affected by different hosts. It is recommended to use end A^- E.coli strains such as DH5α, TOP10 and XL10.
	4. Insufficient lysis	If the bacterial number input is too high, plasmid yield decreases due to incomplete lysis. To optimize results, selecting a suitable bacterial culture volume and an appropriate amount of solvent is recommended.
	5. Inadequate suspension	Proper re-suspension of bacteria is essential for maximizing plasmid yield. The bacterial pellet should be completely suspended until no visible clumps remain.
	6. Incorrect preparation of reagent	Make sure all RNase A solution is added before using Buffer P1. Buffer P2 tends to precipitate over time or at low temperatures, so it should be heated until fully dissolved or placed in a 37°C incubator for 10 min until clear before use. Additionally, confirm that the correct volume of absolute ethanol is added to Buffer PW.
	7. Improper preparation or preservation of bacterial solution	Prolonged shaking of the bacterial culture or the occurrence of lysogeny can lead to plasmid degradation. To maintain plasmid integrity, fresh bacterial cultures should be used whenever possible. The cultivation period should not exceed 16 h, with an optimal range of 12 - 14 h for best results.
	8. Prolonged exposure of the adsorption column to high temperatures or other unfavourable conditions	For long-term storage, it is advisable to keep it at 2 ~ 8°C. Alternatively, adding 100 µl of 3 M NaOH solution can help activate the column membrane for optimal performance before use.
Poor purity	1. Genomic residue	After adding Buffer P2, it must be gently inverted and mixed. When processing multiple samples, the lysis time should not exceed 3 min.
	2. Salt ions residue	It is recommended to add it along the wall of the adsorption column to help reduce the residual salt ion, or rinse twice with Buffer PW.
	3. RNA residue	If RNase A has been mixed with Buffer P1 and left at room temperature for an extended period, its enzyme activity may decline If Buffer P1 is more than 6 months old, add 12.5 µl RNase A (Vazyme #DE111) per 10 ml Buffer P1. To preserve effectiveness, it should be promptly returned to storage at 2 ~ 8°C after use. Additionally, if the bacterial count is too high, the RNase A in Buffer P1 may be insufficient to digest the bacterial RNA. In such cases, reducing the bacterial culture volume is recommended.

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