FastPure Plasmid Mini Kit

DC201

Vazyme

Instruction for Use

Version 23.2

Vazyme

Contents

01/Product Description

Take 1 - 5 ml of overnight bacteria culture and lyse the cells by an optimized SDS-alkaline method. With this kit, the extraction of multiple samples can be completed within 30 min without involving phenol-chloroform extraction or ethanol precipitation. The kit uses a unique spin column to specifically bind plasmid DNA in the solution in a highly efficient manner under high-salt and low-pH conditions and remove proteins, genomic DNA, RNA, and other impurities to the greatest extent. In the end, pure plasmid DNA is eluted from the silica membrane with a low-salt, high-pH eluent. Each column adsorbs up to 35 μg of plasmid DNA. The purified plasmid DNA is ready for use in biological experiments such as enzyme digestion, PCR, sequencing, ligation, transformation, transfection, and common passaging cells experiments.

02/Components

Components	DC201-01 (100 rxns)
RNase A	150 μl
Buffer P1	30 ml
Buffer P2	30 ml
Buffer P3	40 ml
Buffer PW1	60 ml
Buffer PW2	2 × 20 ml
Elution Buffer	20 ml
FastPure DNA Mini Columns	100
Collection Tubes 2 ml	100

RNase A: Degrade RNA in the sample.
Buffer P1: Suspend bacteria. Add RNase A to Buffer P1 before the first use.
Buffer P2: Lyse bacteria (containing SDS/NaOH).
Buffer P3: Neutralizing buffer.
Buffer PW1: Remove proteins and other impurities from plasmids.
Buffer PW2: Remove residual salt ions from plasmids.
Elution Buffer: Elute DNA.
FastPure DNA Mini Columns: Adsorb plasmid DNA.
Collection Tubes 2 ml: Collect filtrate.

03/Storage

Store at 15 ~ 25°C and transport at room temperature.
If ambient temperatures often exceed 25°C, we suggest storing RNase A at 2 ~ 8°C.
After addition of RNase A, Buffer P1 is stable for 6 months when stored at 2 ~ 8°C.

04/Applications

This product is is suitable for 1 - 5 ml of overnight bacterial cultures.
When preparing low-copy plasmids, scale up the volumes of Buffer P1, Buffer P2, and Buffer P3, and increase the volume of bacterial solution to 5 - 10 ml.

05/Self-prepared Materials

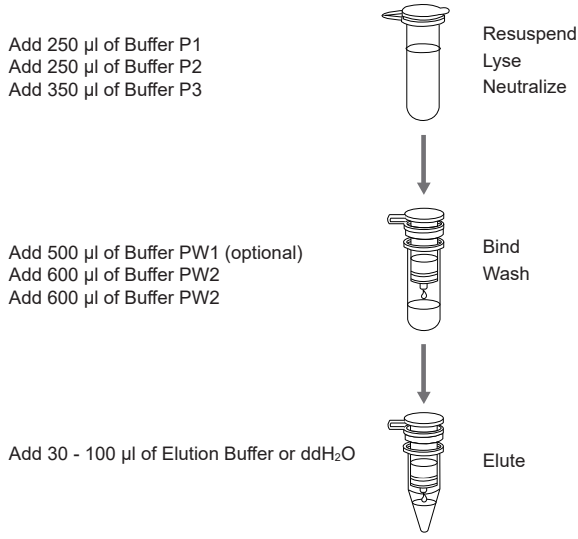
1.5 ml sterile centrifuge tube, absolute ethanol.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. Before the first use, briefly centrifuge RNase A, add all the RNase A supplied in the kit to Buffer P1, and store the mixture at 4°C.
2. Before the first use, add the appropriate volume of absolute ethanol to Buffer PW2 according to the label, and store at room temperature.
3. Buffer P2, Buffer P3, and Buffer PW1 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.
4. Plasmid yield and quality are related to the host bacteria strain, plasmid copy number, and plasmid stability.
5. Buffer PW1 effectively removes residual proteins and must be used for *end*A+ host strains (TG1, BL21, HB101, ET12567, and JM series), which contain high levels of nucleases.
6. When preparing low-copy plasmids, scale up the volumes of Buffer P1, Buffer P2, and Buffer P3, and increase the volume of bacterial solution to 5 - 10 ml.
7. Avoid direct contact with Buffer P2, Buffer P3, and Buffer PW1. Wear gloves when handling the buffers and tighten the bottle caps immediately after use.

07/Mechanism & Workflow



08/Experiment Process

1. Transfer 1 - 5 ml of overnight (12 - 16 h) culture to a centrifuge tube (not provided), and centrifuge at 10,000 rpm (11,500 × g) for 1 min. Discard the culture medium and place the tube inverted on a blotting paper to drain the liquid.
2. Add 250 µl of Buffer P1 (make sure that RNase A has been added) to the centrifuge tube containing the precipitated bacterial cells, and mix thoroughly by pipetting or vortexing.
   ▲Complete resuspension of bacterial cells in this step is critical to plasmid yield. No cell clumps should be visible. Unsuspended cell clumps will hinder lysis, resulting in low plasmid yield and purity.
3. Add 250 µl of Buffer P2 to the mixture from Step 2. Mix by gently inverting the tube 8 - 10 times to completely lyse the cells.
   ▲ Invert gently to mix thoroughly. Vortexing will shear genomic DNA and contaminate the extracted plasmids with gDNA fragments. The solution should become viscous and clear, indicating that the bacterial cells have been fully lysed. 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, so the amount of bacteria should be reduced appropriately.
4. Add 350 µl of Buffer P3 to the mixture from Step 3. Immediately invert the tube gently 8 - 10 times to fully neutralize Buffer P2. At this time, white flocculent precipitates should form. Centrifuge at 12,000 rpm (13,400 × g) for 10 min.
   ▲ Mix immediately by inversion after adding Buffer P3 to avoid localized precipitation, which may result in incomplete neutralization. If the supernatant still contains small white precipitates, centrifuge again before collecting the supernatant.
5. Place FastPure DNA Mini Columns into a Collection Tube 2 ml. Carefully transfer the supernatant from Step 4 to the FastPure DNA Mini Columns with a pipette, taking care not to disturb the precipitates. Centrifuge at 12,000 rpm (13,400 × g) for 30 - 60 sec. Discard the filtrate and place the FastPure DNA Mini Columns back into the Collection Tube.
6. (Optional) Add 500 µl of Buffer PW1 to the FastPure DNA Mini Columns. Centrifuge at 12,000 rpm (13,400 × g) for 30 - 60 sec. Discard the filtrate and place the FastPure DNA Mini Columns back into the Collection Tube.
   ▲ This step is recommended for *end*A+ strains (TG1, BL21, HB101, JM series, ET12567, etc.), which contain high levels of nucleases that may degrade plasmid DNA.
   This step may be skipped for *end*A- strains (DH5α, TOP10, etc.).
7. Add 600 µl of Buffer PW2 (make sure that absolute ethanol has been added) to the FastPure DNA Mini Columns. Centrifuge at 12,000 rpm (13,400 × g) for 30 - 60 sec. Discard the filtrate and place the FastPure DNA Mini Columns back into the Collection Tube.
8. Repeat Step 7.
9. Place the FastPure DNA Mini Columns back into the Collection Tube. Centrifuge the empty column at 12,000 rpm (13,400 × g) for 1 min to completely remove the residual wash buffer.
   ▲ Residual ethanol in the wash buffer will interfere with downstream enzymatic reactions, such as enzyme digestion, ligation, and PCR. Do not skip this step.

10. Place the FastPure DNA Mini Columns in a new sterile 1.5 ml centrifuge tube. Add 30 - 100 µl of Elution Buffer to the center of the spin column membrane. Leave the system at room temperature for 2 min, and centrifuge at 12,000 rpm (13,400 × g) for 1 min to elute the DNA.

    ▲ The elution volume should be no less than 30 µl, as lower volumes decrease the elution efficiency. For maximum yield, pre-heat the Elution Buffer to 55°C to increase elution efficiency. In addition, the eluate can be readded to the FastPure DNA Mini Columns, repeat step 10. If the product is intended for sequencing, elute with ddH_2O and ensure that the pH is within the range of 7.0 - 8.5, as a pH below 7.0 reduces the elution efficiency.

11. Discard the FastPure DNA Mini Columns and store the extracted DNA at -20°C to prevent degradation.

09/FAQ & Troubleshooting

◇ Extraction of low-copy or large plasmids (>10 kb)

For low-copy or large plasmids (>10 kb), increase the number of bacterial cells by using 5 - 10 ml of overnight culture and scale up the volumes of Buffer P1, Buffer P2, and Buffer P3, Incubate Elution Buffer in a 55°C water bath and extend adsorption and elution time to increase extraction efficiency. The other steps remain the same.

◇ Low DNA yield

Plasmid copy numbers: Different copy numbers of plasmid vectors will lead to significant fluctuations in DNA yield. High-copy vectors typically show yield fluctuations within 2 - 3 folds (3 - 16 µg of DNA yield per ml of overnight culture). Large plasmids and expression vectors generally have medium or low copy numbers, yielding 0.5 - 2 µg of DNA per ml of bacterial culture.

◇ Low-copy plasmids: pBR322, pACYC and derivatives, pSC101 and derivatives, SuperCos, and pWE15.

◇ High-copy plasmids: pTZ, pUC, pBS, and pGM-T.

Bacteria strain issues: Plasmid loss occurred during the preservation of the bacteria strain. It is advisable to revive bacteria by streaking onto a growth medium before further culturing to ensure the yield.

Incomplete cell lysis: Bacterial cells should be completely resuspended in Buffer P1/RNase A. Cell clumps cannot be lysed and will result in lower yields.

Reagent preparation errors: If there is any precipitate in Buffer P2, dissolve by heating. You may also have added an incorrect volume of ethanol to Buffer PW2. Control the ethanol concentration at 80%.

Large plasmids: Large plasmids generally have medium or low copy numbers. The bacterial culture volume can be increased to 10 ml to enhance the yield. It is also recommended to pre-heat Elution Buffer to 55°C and repeat elution once.

◇ Genomic DNA contamination

Culture overgrown: Limit bacterial culture to 12 - 16 h of growth.

Lysis issues: Mix the sample by gentle inversion after adding Buffer P2. When processing multiple samples, the lysis time should not exceed 5 min from Buffer P2 addition.

◇ Unsatisfactory results of downstream experiments

Salt ion contamination: Make sure to wash twice with Buffer PW2.

Ethanol contamination: After the final wash with Buffer PW2, increase the column centrifugation time from 1 min to 2 min.

Plasmid degradation: *end*A+ strains (e.g., HB101) or other wild-type strains that contain nucleases in abundance must be washed with Buffer PW1.

Membrane dissociation: The silica membrane may detach from the column during centrifugation when eluting the plasmid. Centrifuge at 10,000 rpm (11,500 × g) for 2 min before transferring the plasmids to a new centrifuge tube.

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