FastPure Plasmid Mini Kit

Catalog # DC201

Version 5.1 Vazyme biotech co., ltd.

1 Introduction

This Kit based on optimized SDS alkaline lysis technology, is applicable for the purification of plasmids DNA from 1 - 5 ml overnight cultures. By using this kit, the extraction of multiple samples can be finished rapidly within 30 min without phenol chloroform or ethanol precipitation. The unique Filtration Column contained in the kit bind the plasmid DNA specifically under high-salt and low-pH condition, maximally removing proteins, genomic DNA, RNA and other impurities. Then the bound plasmid DNA is eluted from silicon membrane in a low-salt and high-pH buffer (Each column can absorb maximally 35 ug of plasmid DNA). The purified plasmid DNA can be directly used for traditional biological experiments: enzyme digestion, PCR, sequencing, ligation, transformation, and transfection of sub-culture cells.

2 Contents

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

RNase A: 50 mg/ml.
Buffer P1: Bacteria suspension solution. Add the provided RNase A solution to Buffer P1 prior to use.
Buffer P2: Bacteria lysis buffer (SDS/NaOH).
Buffer P3: Neutralization buffer.
Buffer PW1: Washing buffer, remove residual protein.
Buffer PW2: Salt-removing buffer.
Elution Buffer: Elute plasmid DNA.
FastPure DNA Mini Columns: Filtration Column of plasmid DNA.
Collection Tube 2 ml : Collection tubes for filtrate.

3 Storage

All regents should be stored at room temperature (15 - 25℃) expect RNase A at -20℃, but RNase A can be transported at room temperature.
▲The buffer forms precipitate easily when stored at low temperature. Dissolve at room temperature for a while or at 37℃ for 10 min if necessary to thaw the precipitate and mixed thoroughly before use.

4 Application

This Kit is applicable for purification of plasmids DNA from 1 - 5 ml overnight cultures. For low-copy plasmid, increasing the volume of Buffer P1, Buffer P2 and Buffer P3 at a ratio and the cultures volume to 5 - 10 ml is necessary to ensure the sufficient yield of plasmids DNA.

5 Additional Materials Required

1.5 ml sterilized tubes, 100% ethanol.

6 Tips

◇Please spin RNase A briefly and add to Buffer P1 before use, store at 4℃.

◇Add moderate ethanol to dilute Buffer PW2 as indicated on tag, store at room temperature.

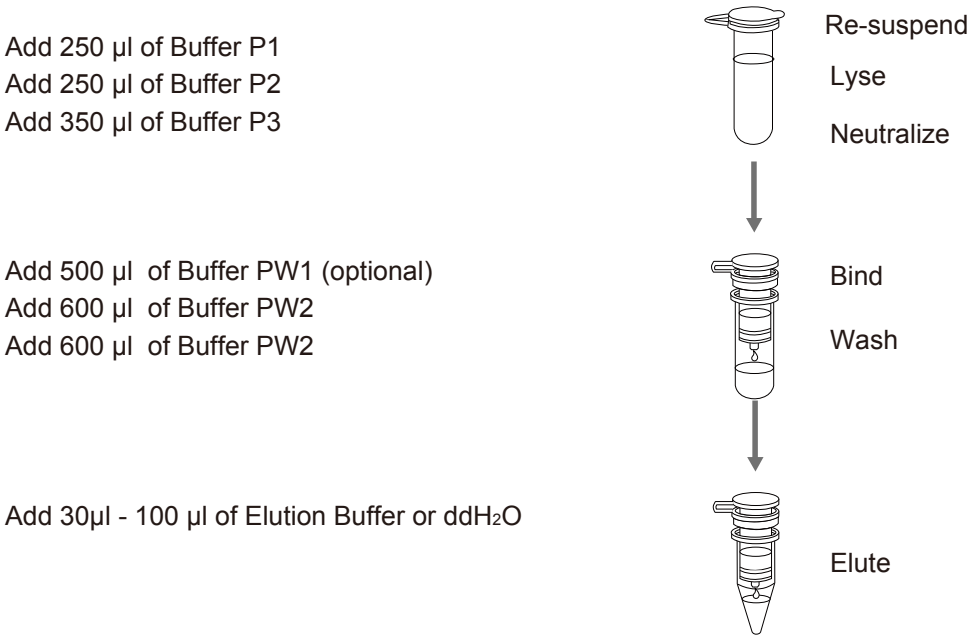
◇The yield and quality of purified plasmid DNA may vary according to strain type, plasmid copy number and plasmid stability.

◇Buffer PW1 (can effectively remove residual protein) must be used when the host strains are endA+ (TG1, BL2, HB101, ET12567, JM) or other strains with high nuclease.

◇For low-copy plasmid, please increase the volume of Buffer P1, Buffer P2 and Buffer P3 at a ratio and increase the cultures volume to 5 - 10 ml.

◇Avoid direct contact to Buffer P2, Buffer P3 and Buffer PW1, wear gloves before performing and immediately close the lid after use.

7 Principle and Procedure



8 Protocol

1. Harvest 1- 5 ml overnight cultured (12 - 16 hr) bacterial cells into a centrifuge tube, centrifuge at 10,000 x g for 1min, discard the supernatant and invert the tube on the absorbent paper to dry.
2. Add 250 µl of Buffer P1 (add RNase A before use), mix thoroughly by vortex or pipetting up and down.
   ▲Attention: Complete resuspension of cell pellet is vital to high yield and purity of plasmid DNA.
3. Add 250 µl of Buffer P2, mix thoroughly by softly inverting the tube 8 - 10 times to assure complete lysis.
   ▲Attention: Gently invert the tube（Do not vortex, avoid shearing of genomic DNA）to get a clear lysate. Restrain the lysis reaction time within 5 min to prevent plasmid breaking. If the lysate is not clear, please reduce bacterial pellet properly.
4. Add 350 µl of Buffer P3, mix gently and thoroughly by inverting the tube 8 - 10 times to neutralize Buffer P2 until a flocculent white precipitate forms, centrifuge at 13,000 x g for 10 min.
   ▲Attention: Immediately invert the tube and mix thoroughly after adding Buffer P3 to avoid localized precipitation that will affect the neutralization. If white precipitate still exists in supernatant, centrifuge again to get a clear supernatant.
5. Insert a FastPure DNA Mini Column into a 2 ml Collection Tube, carefully transfer (by aspirating, avoid disturbing the precipitate) the supernatant from step 4 to the Filtration Column, centrifuge at 13,000 x g for 30 - 60 sec, discard the filtrate and reuse the Collection Tube.
6. (Optional)Add 500 µl of Buffer PW1 to the Filtration Column, centrifuge at 13,000 x g for 30 - 60 sec, discard the filtrate and reuse the Collection Tube.
   ▲Attention: This step is recommended when using endA+ strains (TG1, BL21, HB101, JM series, HB101, ET12567 and their derivatives) which contain a great amount of nuclease that can degrade the plasmid.
   ▲When using endA- strains (DH5α, TOP10 and its derivatives), this step can be omitted.

7. Add 600 μl of Buffer PW2 (with ethanol added in) to the Filtration Column, centrifuge at 13,000 x g for 30 - 60 sec, discard the filtrate and reuse the Collection Tube.
8. Repeat step 7.
9. Centrifuge the empty Filtration Column for l min at 13,000 × g to remove residual washing buffer PW(1,2) .
▲Attention: Residual ethanol in washing buffer PW(1,2) may inhibit subsequent enzymatic reactions, i.e., digestion, ligation, PCR and other experiments. This step can not be omitted.
10. Insert the Column into a clean 1.5 ml microcentrifuge tube, add 30 - 100 μl of Elution Buffer to the center of the Column membrane, incubate at room temperature for 2 min, centrifuge at 13,000 × g for 1min.
▲Attention: The volume of Elution Buffer should be more than 30 μl, because less Elution Buffer will decrease the elution efficacy. Preheating the elution buffer to 65°C, and transferring the filtrate of step10 to the Filtration Columns for twice elution, are helpful to increase the elution efficacy. If using sterile deionized water to elute DNA for next sequencing, make sure the pH is 7.0 - 8.5.
11. Discard the Filtration Columns, store DNA at -20℃.

9 Troubleshooting

1. Extraction of plasmid with low copy number or long fragment (>10kb)

When extracting the plasmid with low copy number or long size than 10kb, it is recommended to increase the volume of Buffer P1, Buffer P2 and Buffer P3 at a ratio as well as the cultures volume to 5 -10 ml. Preheating the elution buffer to 55°C, and prolonging the binding and washing time, can help to improve the extraction efficiency.

2. Low DNA yield

Copy number of plasmid: The yield of plasmid vary according to copy number. 1ml of overnight culture with high copy number typically produce 3 μg to 16 μg of plasmid DNA. By contrast, 1 ml of overnight culture with medium, low copy number (which are common for long plasmid or expressing plasmid) normally output 0.5 μg - 2 μg of plasmid DNA.
◇Low copy-number plasmid: pBR322, pACYC, pSC101 and their derivatives, SuperCos, pWE15.
◇High copy-number plasmid: pTZ, pUC, pBS, pGM-T.

Strain: The plasmid may lost during strain storage; it is recommended to take streak plate method for yield stability.
Poor cell lysis: The bacterial should be re-suspended thoroughly in Buffer P1/RNase A; cell clumps owing to insufficient lysis will decrease the yield.
Incorrect regent preparation: Dissolve precipitate in Buffer P2; added correct volume of ethanol to Buffer PW2 to keep the finally concentration of ethanol at 80%.
Long-fragment plasmid: As low and medium copy number are common in long-fragment plasmid, it is expected to increase culture volume to 10 ml to improve the yield of plasmids DNA.
Preheat the Elution Buffer to 55°C and elute twice.

3. Genomic DNA contamination

Long culture time: Do not incubate cultures more than 16hr at 37°C.
Lysis: Invert the tube gently after adding the Buffer P2; ensure the lysis time (counting since Buffer P2 first added) does not exceed 5 min when extracting multiple samples.

4. Unsatisfactory downstream result

Salt pollution: Ensure that wash the pellet with Buffer PW2 twice.
Ethanol pollution: Increase the centrifuge time to 2 min after the second washing with buffer PW2.
Plasmid degradation: When endA+ strains (i.e., HB101 and other wild strains with high nuclease) were adopted, Buffer P1 must be used.
Membrane falling off: Silicon membrane is easily to fall off when centrifuge to elute plasmid. Transfer the plasmid to a new tube after centrifuging at 10,000 × g for 2 min.