For research use only Version Number：2.0-2008

General Plasmid Mini Kit

For purification of plasmid DNA

Kit content	DE-01001	DE-01002	DE-01003
	50T	100T	250T
Buffer S1	12.5 ml	25 ml	62.5 ml
Buffer S2	12.5 ml	25 ml	62.5 ml
Buffer S3	17.5 ml	35 ml	87.5 ml
Buffer PW	25 ml	50 ml	125 ml
Buffer WB2	15 ml	30 ml	75 ml
Buffer EB	10 ml	20 ml	50 ml
DNA-Only Column	50	100	250
Manual	1	1	1

Introduction

The Company's General plasmid mini Kit adopts DNA-only purification column technology and efficient SDS lysis method to obtain high-quality Plasmid DNA from bacteria Based on the company's unique DNA-only purification column and unique formula reagent, can maximize the removal of RNA, without adding RNA enzyme, can remove RNA effect, so that the laboratory from RNA enzyme pollution but also can efficiently remove impurities in protein ions and other organic compounds in bacteria The actual plasmid DNA yield and purity were related to the host bacteria species and culture conditions bacterial lysis and plasmid copy number (high or low copy) and other factors.

This kit is suitable for plasmid DNA extraction of Gram-negative bacteria. The obtained plasmid DNA has high purity, no RNase and very low ion content, which can meet the requirements of various conventional experiments, such as invertase PCR library construction and sequencing (ddH2O eluting plasmid DNA is recommended).

Storage and Stability

The kit can be stored for 12 months at room temperature (15–25℃) under dry conditions. If longer storage can be placed in 2-8℃.If stored at low temperature, the solution is easy to precipitate. Before use, the solution in the kit must be placed in room temperature for a period of time. If necessary, it can be preheated in 37℃water bath for 10 minutes to dissolve the precipitate and mixed before use

Plasmid DNA extraction rate

The amount of plasmid DNA extracted was related to the concentration of bacterial culture copy number strain culture conditions. The plasmid DNA obtained had high purity, $OD_{260}/_{280}$>1.7

plasmid	Culture Condition	Types of plasmid	Yield(1ml)
Low copy	LB/37℃/16hr Culture Condition	pBR322、pACYC、SuperCos、pWE15、pSC101	0.2-1 μg DNA
High copy		pUC、pBS、pTZ、pGEM	2-8 μg DNA

Warnings and Precautions

- The amount of bacterial solution should not be too much. OD_{600}=2.0-3.0 is recommended, and the amount should not exceed 5ml, otherwise the yield and purity of extracted plasmid DNA will be affected.
- Before use, carefully check whether there is precipitation in Buffer S2 and Buffer S3. If there is precipitation, please dissolve it in 37℃ and mixed before use.
- Before using the kit, be sure to check whether anhydrous ethanol has been added to Buffer WB2.
- Elution volume: should not be less than 50μl, otherwise it will affect plasmid DNA yield.
- All the experimental steps were carried out at room temperature (15-25℃), including centrifugation steps by bench centrifuge at room temperature.

Detection of DNA concentration and purity

- The quality of the plasmid DNA obtained depends on a variety of factors during the operation. The concentration and purity of DNA can be determined by agarose gel electrophoresis and ultraviolet spectrophotometer.
- DNA with an OD_{260} value of 1 corresponds to about 50μg/ml of double-stranded DNA.
- DNA $OD_{260}/_{280}$ not less than 1.7 If deionized water is used for elution, ensure that the pH is in

the range of 7.0-8.5. A pH below 7.0 will reduce elution efficiency and the $OD_{260}/_{280}$ ratio will be low.

Procedure

Please add anhydrous ethanol to Buffer WB2 before use. Please refer to the label on the bottle for the volume added.

1. Take **1-5ml** of culture broth for 16-20hr and add it to the centrifuge tube, centrifuge it at 12,000rpm(~13,400×g) for **1min**, and try to suck up the residual supernatant (if there is too much broth, the bacterial precipitation can be collected into a centrifuge tube for several times).
   Note: It is recommended that the bacteria solution OD_{600}=2.0-3.0, and the bacteria should not be excessive, otherwise it will cause incomplete lysis of the bacteria, resulting in low extraction amount and purity. It is recommended to use 2ml centrifuge tube, which will make bacterial precipitation and Buffer S1 more easily mixed than 1.5ml centrifuge tube in subsequent operations
2. Add **250μl** Buffer S1 into the centrifuge tube with the bacteria precipitation, use a pipette or vortex oscillator to thoroughly suspend the bacteria precipitation, and thoroughly suspend the bacteria precipitation until the bacteria clumps can not be seen.
   Note: If there are incomplete mixed bacteria, it will affect the cracking, resulting in low extraction rate and purity
3. Add **250μl** Buffer S2 into the centrifuge tube and gently turn it up and down **6-8** times to fully lysis the thallus until the solution is homogeneous jelly.
   Note: gently and slowly turn over, do not shake violently, so as not to interrupt genomic DNA and cause the extracted plasmid to be mixed with genomic DNA fragments. At this time, the strain of bacterial liquid should be sticky, the time should not exceed 5min, so as not to damage the plasmid.
4. Add **350μl** Buffer S3 into the centrifuge tube, immediately upside down **6-8** times, fully mixed, then white flocculent precipitate will appear at 12,000rpm(~13,400×g) for **10min** centrifugation.
   Note: Buffer S3 should be mixed upside down immediately after addition to avoid local precipitation. Put Buffer S3 on ice for pre-cooling, or put it after adding S3 and mixing for 2min to improve output.
5. Carefully transfer the supernatant to the DNA-only Column, centrifuge at 12,000rpm(~13,400×g) for 1min, and discard the waste liquid in the collection tube.
   Note: Try not to absorb the precipitate. If there is small white precipitate in the supernatant, centrifuge the supernatant again and add it to the centrifugal column.
6. Add **500μl** Buffer PW into the centrifugal column, centrifuge at **3,000rpm(~900×g)** for **1min** at low speed to discard the waste liquid in the collection tube.
7. Add **700μl** Buffer WB2(**check again whether anhydrous ethanol has been added to Buffer WB2**) into the centrifugal column, centrifuge at 12,000rpm(~13,400×g) for **1min**, and discard the waste liquid in the collection tube.
8. Repeat Step 7.
9. The centrifuge column was put back into the collecting tube and centrifuged in 12,000rpm(~13,400×g) empty tube for 2min to remove the residual Buffer WB2 in the centrifuge column.
   Note: Ethanol residue in Buffer WB2 will affect subsequent enzyme reaction (enzyme digestion PCR, etc.) experiments. If necessary, the cover of the centrifugal column can be opened and placed at room temperature for several minutes to dry the residual washing liquid
10. Place the centrifuge column in a clean centrifuge tube, drop **50-200μl** Buffer EB into the middle of the silica gel membrane (do not add the eluent to the pressure ring, otherwise a large volume of eluent will be lost), place at room temperature for **2min** and centrifuge at 12,000 rpm (~13,400×g) for **1min** to collect the DNA solution.
    Note: In order to increase the amount of DNA recovered, the eluting solution obtained by centrifugation can be added back to the centrifugal column. Repeat Step 10 to preheat the eluting buffer EB at 65-70℃ to increase the amount of DNA recovered.
    The minimum elution volume of 50μl should not be less than 50μl in consideration of DNA recovery and concentration. If the elution volume is too small, it will affect plasmid DNA yield. In order to improve the concentration of purified DNA, under the premise of sacrificing part of The DNA yield, the eluting volume can be appropriately reduced, such as using 30μl elution system, in order to obtain higher concentration of DNA.