Biospin Plasmid DNA Mini Extraction Kit

BIOER

Kit Components

Cat#	BSC01S1	BSC01M1	BSC01L1
Component	**50T**	**100T**	**250T**
Resuspension Buffer	12.5 mL	25 mL	62.5 mL
Lysis Buffer	12.5 mL	25 mL	62.5 mL
Neutralization Buffer	17.5 mL	35 mL	87.5 mL
Wash Buffer	15 mL (add 60 mL ethanol before use)	15 mL× 2 (add 60 mL ethanol before use)	37.5 mL× 2 (add 150 mL ethanol before use)
Elution Buffer	10 mL	20 mL	50 mL
RNase A	50μL	100μL	250μL
Spin Column	50 tubes	100 tubes	250 tubes
Handbook	1copy	1copy	1copy

Storage and transportation

◆ The kit should be stored at room temperature(15~25℃), but the RNase solution should be stored at 2~8℃. The kit can be stored for up to 18 months by this method. After addition of RNase solution, Resuspension Buffer should be stored 2~8℃.

◆ The kit can be transported at room temperature.

Introduction

The kit provides a fast, simple, and cost-effective plasmid miniprep method for routine molecular biology laboratory applications. Plasmid DNA can be purified from 1-5mL of overnight cultures of E. coli. The DNA isolated by this kit is ready for downstream applications such as restriction enzyme digestion, sequencing, PCR/Real-time PCR and other downstream experiments.

Technical Information

Method	Work time	Column volume	Column yield	Elution recovery	Plasmid DNA length	Culture volume
Spin column	25 min for 24 samples	750μL	20μg DNA	≥99%	≤10kB	1~5mL high-copy plasmid

Apparatus and materials to be prepared by the user

* Sterile 1.5mL micro centrifuge tubes
* 10μL/100μL/1000μL tips
* microcentrifuge capable of 14,000 ×g
* Absolute ethanol
* Vortex mixer

Important notes

1. The RNase solution should be all added into the Resuspension Buffer before use, mix and store at 2-8℃.
2. Add 60mL absolute ethanol to Wash Buffer before use and mix well.
3. If the Lysis Buffer and Neutralization Buffer precipitated, it should be redissolve by warming to 37℃. Please not vortex Lysis Buffer acutely.
4. Please close the lid immediately after using Lysis Buffer so as to avoid acidification.
5. The kit can extract high-quality plasmid DNA from 1-5mL E.coli overnight cultured.
6. The suitable volume is 50μL for Elution Buffer; user can adjust its volume if necessary.

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Procedure

1. Add 1-1.5mL cultured bacteria to 1.5mL micro centrifuge tube.
2. centrifuge at 10, 000rpm(8,000 ~ 10,000×g)for 30s,and discard the supernatant. Step 1th and 2th could be repeated for more than one time to collect enough cells.
3. Resuspend pelleted bacterial cells in 250μL Resuspension Buffer and No cell clumps should be visible after resuspension of the pellets.
4. Add 250μL Lysis Buffer and gently invert the tube 4-6 times to mix. Do not vortex, as this will result in shearing of genomic DNA. Do not allow this step for more than 5 minutes.
5. Add 350μL Neutralization Buffer and gently invert the tube 4-6 times to mix. The solution should become cloudy and no local precipitate should be visible.
6. Centrifuge for 10minutes at 13,000rpm (> 14,000×g) until a compact white pellet form.
7. Apply the supernatant to the Spin column and centrifuge for 30-60 seconds at 6,000×*g*. Discard the flow-through.
8. Add 650μL Wash Buffer to the Spin column and Centrifuge for 30-60 seconds at 12,000×*g*. Discard the flow-through.
9. Repeat step 8th once.
10. Centrifuge for an additional 1 minute at 12,000×*g* and transfer the Spin column to a sterile 1.5mL micro centrifuge tube. Recommend to centrifuge according to this step; otherwise, there will be residual liquid in the column.
11. Add 50μL Elution Buffer, ddH_2O or TE Buffer to the Spin column and let it stand for 1 minute at room temperature. The volume of elution buffer could be adjusted according to needs.
12. Centrifuge for 1 minute at 12,000×*g*. The buffer in the micro centrifuge tube contains the plasmid DNA.
13. The purified plasmid DNA can be used directly for kinds of downstream molecular biological experiments. Store at -20°C if not used immediately.

Troubleshooting

Get on plasmid DNA

If the plasmid DNA is not found in elution buffer · please check whether the ethanol had been added to Wash Buffer according to the volume be marked on bottle label.

Low plasmid DNA yields

1) Please make sure that the bacteria was cultured in the right way.

2) The bacteria cells should be resuspented completely.

3) Incubate the Elution Buffer in 30 ~ 60°C, it will increase the yields.

Absorbance problem

1) Absorbance is the difference from sample and criterion, please use the Elution Buffer to adjust to zero value and dilute the sample.
2) If the ratio of OD260/ OD230 is low, wash the spin column for one more time.
3) In the case of low ratio of OD260-320/ OD280-320, there is protein contamination, please add Neutralization Buffer, and then centrifuge buffer with sufficient rotating speed, thus to make precipitation compact; be careful to pipette supernatant so as to avoid pipette precipitation.
4) If the ratio of OD260-320/OD280-320 is high · add RNase A to Resuspension Buffer (100μg/mL).

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Electrophoresis problem

1） If there is genomic DNA in the result. Please invert the tube gently (step4 and 5).

2） If there is RNA in the result, add RNase A to Resuspension Buffer (100μg/mL).

DNA Analysis

⊕ **Absorbance analysis**

Get some plasmid DNA，diluted in a advisable factor with elution buffer.

Survey the OD260, OD280 and OD320

Expressions: concentration（μg/mL）= 50×OD260×dilution fact

Target: 2.0≥OD260-320/ OD280-320≥1.8

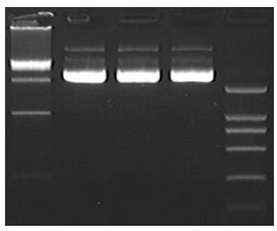
Notice: 1.0≥OD260≥0.1, the result of ratio is much reliable.

⊕ **Agarose Gel Analysis**

0.8 ~ 1% Agarose gel

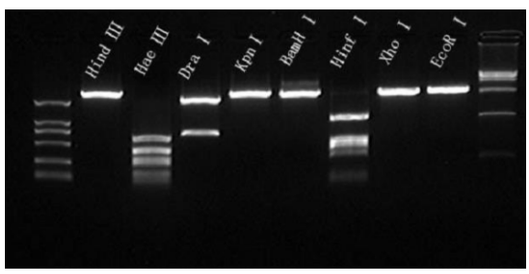
Example 1: Plasmid DNA electrophoresis

DL15, 000 DL2, 000



Example 2: Enzymatic reactions analysis

DL2, 000 DL15, 000



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