常见问题及对策

没有提取到质粒

如在洗脱后，发现溶液中没有质粒 DNA，请检查是否按 Wash Buffer 瓶身标签标明的体积加入了无水乙醇。

质粒提取率低

1） 请先确认培养的细菌，排除培养过程中的杂菌污染、质粒丢失等原因。

2） 加入重悬液后应使菌体沉淀充分悬浮分散。

3） 在洗脱前将 Elution Buffer 于 30～60℃温育，可提高提取效率。

吸光度测量结果问题

1） 吸光度测量的是未知样品与调零标准之间的相对吸光度，所以请用与洗脱液体相同的液体，对测量样品进行稀释和调零。

2） OD_{260}/ OD_{230} 比值偏低，可用 Wash Buffer 多一次对离心柱进行洗涤。

3） 如 $OD_{260-320}$/ $OD_{280-320}$ 比值偏低，说明有蛋白质污染。应在加入 Neutralization Buffer 后，以足够高的转速离心，使沉淀紧密，并小心地吸取上清液，避免吸入沉淀。

4） 如 $OD_{260-320}$/ $OD_{280-320}$ 比值偏高，说明有 RNA 污染，请在重悬液中加入 RNase A 至终浓度 100μg/ml。

电泳结果问题

1） 有基因组条带。在加入 Lysis Buffer 及 Neutralization Buffer 后，颠倒离心管应柔和，以避免基因组 DNA 受到剪切。

2） 有 RNA 污染。请在重悬液中加入 RNase A 至终浓度 100μg/ml。

核酸的检测分析及图例见英文说明书

Biospin Plasmid DNA Extraction Kit

Biospin 质粒 DNA 小量提取试剂盒

Cat# **BSC01M1**

TECHNICAL SUPPORT:

For technical support, please dial phone number ： 0086-571-87774567-5278 or 5211, or fax to 0086-571-87774303

email to reagent@bioer.com.cn.

Website: www.bioer.com.cn

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, ddH2O 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℃ 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℃，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 OD_{260}/ $OD_{230}$is low，wash the spin column for one more time.
3) In the case of low ratio of $OD_{260-320}$/ $OD_{280-320}$, 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 $OD_{260-320}$/ $OD_{280-320}$is high，add Rnase A to Resuspension Buffer (100μg/ml)

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 anlysis

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

Survey the OD_{260} ,OD_{280} and OD_{320}

expressions：concentration（μg/ml）＝50×OD_{260}×dilution fact

target： $2.0 \geq OD_{260-320}/ OD_{280-320} \geq 1.8$

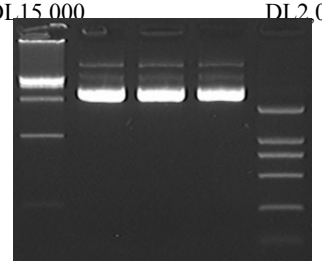
Notice: $1.0 \geq OD_{260} \geq 0.1$, the result of ratio is much reliable.

⊕ Agarose Gel Analysis

0.8～1％ Agarose gel

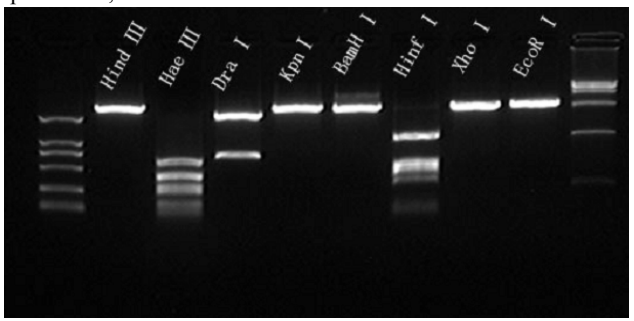
Example 1：plasmid DNA electrophoresis

DL15,000 DL2,000



Example 2：**enzymatic reactions analysis**

Example：DL2,000 DL15,000



试剂盒组成(100T)

成分	数量
Resuspension Buffer	25ml
Lysis Buffer	25ml
Neutralization Buffer	35ml
Wash Buffer	60ml
Elution Buffer	20ml
RNase solution	1
Spin columns	100
说明书	1 份

储存与运输

- 本试剂盒中除 RNA 酶保存于 2～8℃以外，其余试剂保存于室温（15～25℃）。所有试剂如果按上述方法保存，可以稳定保存 18 个月。如已将 RNA 酶全部移入重悬液，请将重悬液保存于 2～8℃。
- 可在常温下运输。

介　绍

本试剂盒提供了一个从大肠杆菌中提取高质量质粒 DNA 的简单、快速、有效的技术，适应于从 1-5ml 过夜培养的大肠杆菌中提取质粒，提取的质粒 DNA 可以直接应用到限制性酶切、测序和 PCR/Real-time PCR 等各类下游分子生物学实验。

基本技术参数

提取方法	操作时间	离心柱容积	单柱最大结合量	洗脱回收率	样本用量
离心柱	25 分钟内完成 24 个样本	750μl	20μg DNA	≥99%	1～5ml 高拷贝质粒细菌

需要的配套设备和材料

* 无菌1.5ml离心管
* 离心机（最大转速＞14,000g）
* 漩涡振荡器
* 各种规格移液器和无菌移液器吸头
* 无水乙醇

Important notes

1. The RNase solution should be all added into the Resuspension Buffer before use, mix and store at 2-8℃.“
2. Add ethanol (as the volume be marked on bottle label) to Wash Buffer and mix well.
3. If the Lysis Buffer and Neutralization Buffer precipitated, it should be Redissolve by warming to 37°C. 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 50ul for Elution Buffer, user can adjust its volume if necessary.

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**

Kit Components(100T)

Component	Amount
Resuspension Buffer	25ml
Lysis Buffer	25ml
Neutralization Buffer	35ml
Wash Buffer	60ml
Elution Buffer	20ml
RNase solution	1
Spin columns	100
Handbook	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.

Apparatus and Materials to Be Supplied by the User

* sterile 1.5ml micro centrifuge tubes * 10μl/100μl/1000μl tips
* microcentrifuge capable of 14,000 × g * Absolute ethanol * Vortex mixer

重要提示

1) 第一次使用时请将 RNA 酶溶液全部移入重悬液，并将重悬液保存于 2～8℃。
2) 使用前请按 Wash Buffer 瓶身标签标明体积加入无水乙醇，并将其混匀。
3) 使用前检查 Lysis Buffer 和 Neutralization Buffer 是否有沉淀，如果有沉淀用 37℃温浴溶解。不要剧烈摇晃 Lysis Buffer。
4) Lysis Buffer 用好后立即盖好盖子以防止酸化。
5) 本试剂盒可从 1～5ml 过夜培养的细菌中提取高质量质粒 DNA。
6) 本试剂盒最适 Elution Buffer 体积为 50μl，Elution Buffer 用量可根据客户实验具体情况灵活调整。

操作过程

1. **将 1～1.5ml 过夜培养的细菌菌液加入 1.5ml 离心管中。**
2. **于 10，000rpm（8,000～10,000×g）离心 30 秒，并弃去上清液。**
   如有需要，可多次重复步骤 1、2，以收集更多细菌菌体。但勿过量，以免影响提取质粒的质量。
3. **加 250μl Resuspension Buffer，重悬细菌体沉淀。**
   重悬后应该没有细菌团块。
4. **加 250μl 细胞 Lysis Buffer，轻柔颠倒 4～6 次。**
   不要剧烈振动，以防止基因组 DNA 被剪切。注意不要让反应持续超过 5 分钟。
5. **加 350μl Neutralization Buffer，立即轻柔颠倒离心管 4～6 次。**
   溶液应该出现絮状物，但不会出现局部沉淀。
6. **于 13,000rpm（＞14，000×g）离心 10 分钟。**
   如离心机转速不够可延长离心时间，直至形成紧密的白色沉淀。
7. **将步骤 6 离心后得到的上清液转移到 Spin column 内。于 6,000rpm（≤6,000×g）离心 1 分钟，并弃去接液管内液体。**
8. **向 Spin column 内加 650μl Wash Buffer，于 12，000g 离心 30～60 秒，并弃去接液管内液体。**
9. **重复第 8 步一次。**
10. **再次于 12，000g 离心 1 分钟，然后将 Spin column 转移到无菌的 1.5ml 离心管中。**如不进行该步离心，则无法保证离心柱内残液被彻底清除。
11. **向 Spin column 内加 50μl Elution Buffer、去离子水或 TE 溶液，并于室温静置 1 分钟。**可根据实验的实际需要决定洗脱液用量。
12. **于 12，000g 离心 1 分钟，1.5ml 离心管内溶液中含有质粒 DNA。**
13. **提取的质粒 DNA 可直接用于各类下游分子生物学实验，如果不立即使用，请保存于-20℃。**