吸光度测量结果问题

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。

如何计算提取率

1) 由于回收前样品中，往往含有非目的 DNA 片段、引物、dNTP 等，所以不能用测样品回收前后吸光度的的方法计算回收率。
2) 可将回收前后 DNA 片段一起电泳，使用凝胶成像系统拍照后，用配套的软件进行电泳条带灰度对比。
3) 注意，电泳条件及拍摄条件将对灰度对比结果造成很大影响，请仔细操作，以减小误差。

DNA 片段长度与提取率

本试剂盒的对于 50bp 以下的 DNA 片段无提取能力，该设计是为了有效去除 PCR 反应中的引物。

核酸的检测分析及图例见英文说明书

Biospin PCR Purification Kit

Biospin PCR 产物纯化试剂盒

Cat# BSC03M1

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

Kit Components(100T)

Component	Amount
Binding Buffer	20ml
Wash Buffer	30ml
Elution Buffer	20ml
Spin column	100
Handbook	1copy

Storage and Transportation

- The Kit should be stored dry at room temperature(15~25℃), The kit can be stored for up to 18 months if all components are kept in the manner above.
- The kit can be transported at room temperature.

Introduction

The Kit provides a simple, rapid and effective method for purification of DNA fragments from PCR or enzymatic reaction. DNA fragments ranging from 60bp to 10kb can be purified. The yield of DNA with size lower than 100bp is 23~95%, while the yield of DNA with size from 0.1kb to 10kb is 90~97%. Purified DNA can be used directly for kinds of downstream molecular biological experiments such as cloning, sequencing, restriction enzyme digestion, PCR/real-time PCR and so on.

Technical Information

Method	Work time	Column volume	DNA size range	Elution recovery	Sample volume
Spin column	6 min for 2 samples	750μl	60bp～10kb	≥99%	Up to 100μl reaction

Apparatus and Materials to Be Supplied by the User

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

重要提示

1. 使用前按 Wash Buffer 瓶身标签标明体积加入无水乙醇，并将其混匀。
2. Binding Buffer 用好后立即盖好盖子。
3. 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

操作步骤

1. **将 PCR 反应产物或其它酶促反应物移入 1.5ml 离心管中。**
2. **按 PCR 反应产物体积的 2 倍加入 DNA Binding Buffer。**
   每次加入的 DNA Binding Buffer 最大体积不宜超过 200μl。
3. **将混合液全部转移到 Spin column 内。**
4. **于 6，000g 离心 1 分钟，并弃去接液管内液体。**
5. **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。**
6. **重复第 5 步一次。**
7. **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。
8. **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。
9. **于 12，000g 离心 1 分钟，1.5ml 离心管内溶液中含有目的 DNA 片段。**
10. **提取的 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℃。**

常见问题及对策

没有提取到 DNA 片段

如在洗脱后，发现洗脱液中没有 DNA 片段，请检查是否按 **Wash Buffer** 瓶身标签，在 **Wash Buffer** 中加入了无水乙醇；

提取率低

1. 结合液为酸性缓冲液，由于意外原因导致其 pH 值升高将影响提取得率。请加入 0.1 倍体积的 3M 乙酸钾（pH5.0）。
2. 在洗脱前将洗脱液于 30～60℃温浴，可提高提取效率。

试剂盒组成(100T)

成分	数量
Binding Buffer	20ml
Wash Buffer	30ml
Elution Buffer	20ml
Spin column	100
说明书	1 份

储存与运输

- 试剂存于室温（15～25℃）。所有试剂如果适当保存，可以稳定保存 18 个月。
- 可在常温下运输。

介　绍

本试剂盒提供一个从 PCR 反应产物及其它酶促反应物中，提取纯化高质量 DNA 的简单，快速，有效的技术，适合的提取 DNA 片段长度为 60bp~10kb，小于 100bp 片段提取效率为 23~95%，0.1～10kb 片段提取效率为 90~97%，纯化 DNA 可以应用到克隆、测序、限制性酶切和 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	提取 DNA 片段范围	洗脱液回收率	样本用量
离心柱	6 分钟内完成 2 个样本	750μl	60bp～10kb	≥99％	最大 100μl 酶促反应物

需要的配套设备和材料

* 无菌1.5ml离心管　　* 各种规格移液器和无菌移液器吸头

* 离心机(最大转速>14,000g)　　* 漩涡振荡器　　* 无水乙醇

Important notes

1. Add ethanol (as the volume be marked on bottle label) to Wash Buffer and mix well
2. Close the lid after using the Binding Buffer as soon as possible.
3. The suitable volume is 50ul for Elution Buffer, user can adjust its volume if necessary.

Procedure

1. **Transfer the PCR or enzymatic reaction product to 1.5ml microcentrifuge tubes.**
2. **Add 2 volumes of Binding Buffer to 1 volume of the PCR or enzymatic reaction product and vortex.**

   The maximal volume added every time should not exceed 200μl .
3. **Apply the mixture to the Spin column by decanting or pipetting.**
4. **and centrifuge for 1min at 6,000×*g*. Discard the flow-through.**
5. **Wash the Spin column by 650μl Wash Buffer in centrifuging for 30-60 seconds at 12,000×*g*. Discard the flow-through.**
6. **Repeat Step 5th once.**
7. **Centrifuge for an additional 1 minute at 12,000×*g* and transfer the Spin column to a sterile 1.5ml microcentrifuge tube.** Recommend to centrifuge according to this step; otherwise, there will be residual liquid in the column
8. **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.
9. **Centrifuge for 1 minute at 12,000×g. The buffer in the microcentrifuge tube contains the DNA.**
10. **The purified plasmid DNA can be used directly for kinds of downstream molecular biological experiments. Store at -20℃ if not used immediately.**

Troubleshooting

No recovery

If the DNA fragment 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 recovery

1） The extraction buffer is acidic buffer，if the pH increases after gel excated, it will leads to inefficient DNA binding. Pleas add 0.1volume 3M sodium acetate（pH5.0）.

2） Incubate the Elution Buffer in 30～60℃， it will increase the yields.

Absorbance problem

Absorbance is the difference from sample and criterion，please use the Elution Buffer to adjust zero value and dilute the sample.

How to calculate yield

1) Because there are usually nonpurpose DNA, primer and dNTP before sample purification, please do not use method of absorbance analysis to calculate yield.

2) User can electrophoreses DNA both before purification and after purification, and then take photo by imaging system, thus to compare brightness of nucleic acid belt by using equipped software.

3) Pay close attention to operation to reduce error, for electrophoresis and photo condition will affect comparison result

The size of DNA and yield

This kit is available for DNA whose size is larger than 50bp, thus to remove primer of PCR product efficiently.

Analysis DNA

⊕Absorbance anlysis

Get some 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.

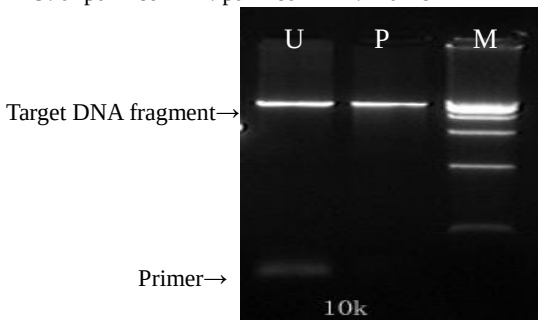
⊕Agarose Gel Analysis

0.8～1% Agarose gel

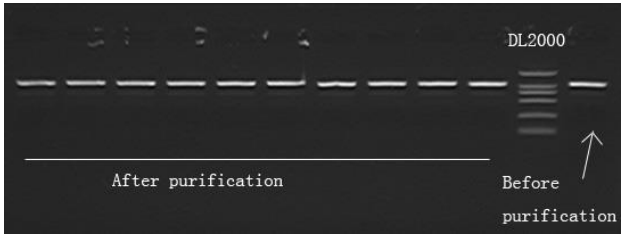
Example 1：

According to the absorbance and agarose gel analysis，the impurity had been discarded.

U: unpurified P: purified M: Marker



Example 2：



Example3：Elution Volume versus DNA Yield