弃去接液管中液体。

13 重复步骤12的操作一次。

14 再次将Spin column于10,000 x g 离心1分钟，并将Spin column转移至一个新的1.5ml离心管。

15 向 Spin column 中加入100μl Elution Buffer，并于室温温育1分钟。

16 于10,000 x g 离心1分钟，并弃去Spin column。1.5ml离心管中液体含有DNA。

17 提取的DNA可直接用于各种下游应用实验，如不立即使用，请于-20℃保存。

## 常见问题及对策

**1 细菌提取的种类**

A 绝大部分细菌,包括革兰阳性细菌和革兰阴性细菌,可有效破碎细菌细胞结构。

**2 RS buffer 有混浊怎么办?**

A 请充分摇匀。

**3 RNase A 是否一定需要?**

A 建议使用.但具体情况视实验目的而定,如果提取的基因组DNA 进行PCR 扩增,酶切等普通分子生物学下游实验,则不需加入,如果对DNA 纯度要求比较高,可以加入一定量的RNase A 以保证RNA 被充分消化。

**4 提取过程中有有机溶剂吗?**

A 不需要。

**5 提取的细菌基因组片段大小是多少?**

A 本试剂盒提取的基因组 DNA 片段大小在 20-150kb 之间。

**6 提取的基因组 DNA 量很少是怎么回事?**

A 需要注意的问题主要有以下几点。

1) 请确认细菌处于生长旺盛的对数生长期;
2) 确认 EL Buffer 中已经加入了溶菌酶并充分混匀;
3) 加入 EL Buffer 后,将离心管底部的细菌团块充分打散.保证足够温育时间;
4) 确保提取的其它过程严格按照说明书的要求进行;

**7 提取的细菌数量在什么范围内?**

A 一般情况下,液体培养基培养的细菌在对数生长期时的细菌数量约为 $10^7$~$10^8$/ml 之间,最大提取的细菌数量为 $5\times10^9$.如果使用固体培养基培养请进行细胞计数。细菌数量较少时请注意富集。

## 核酸的检测分析及图例见英文说明书

# Biospin Bacteria Genomic DNA Extraction Kit

# Biospin 细菌基因组 DNA 提取试剂盒

Cat# BSC12M1


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 |
|---|---|
| Proteinase K | 1 ml |
| EL Buffer | 10ml |
| RS Buffer | 10 ml |
| GA Buffer | 20 ml |
| BA Buffer | 20ml (add 21ml ethanol before use) |
| G Binding Buffer | 50ml |
| Wash Buffer | 42ml (add 63ml ethanol before use) |
| Elution Buffer | 20 ml |
| Spin column | 100 |
| Handbook | 1 copy |

## Storage

- The Proteinase K is to be stored at 2-8℃,others at 15-25℃.
- All reagents, when stored properly, are stable for 18 months.

## Introduction

The Kit provides a very simple, fast and economic way for the isolating of pure high–molecular-weight genomic DNA from all kinds of bacterial, including Gram-NegativeBacterial and Gram-Positive Bacterial, adopting the Genomic DNA Buffer Set. The simplepurification procedure, based on the remarkable selectivity of Biospin membrane, allows isolationof high yields of pure genomic DNA in less than 1 hour. It requires no expensive equipment, involves only few steps, and completely avoids the use of toxic and hazardous reagents such as phenol and chloroform. In general, the Kit can get at most 30ug genomic DNA from $5\times10^9$ Bacterial cells.

The pure DNA can be applied extensively in PCR/Real time PCR, sequencing, Southern blot, mutant analysis, SNP and the others.

## Principle

The structure of bacterial cells is destroyed completely by EL Buffer, DNA in the sample is liberated under the cooperation of RS Buffer, GA Buffer and PK solution. After lysis,. Simple processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cationsgt Released DNA is bound exclusively and specifically to the Biospin membrane in presence of a Binding Buffer under appropriate salt iron and pH conditions. Denatured protein and other contaminants are removed by several washing procedures. The DNA is then eluted from the membrane with the Elution Buffer.

## 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）
* 无水乙醇
* 漩涡振荡器

## 注意事项

1. **对于革兰氏阴性细菌（可选做此步骤）**，第一次使用前，称取315mg溶菌酶，加入EL Buffer,左右摇晃10-15次，大约30秒，直至溶液清亮，2～8℃保存。
   **对于革兰氏阳性细菌**：第一次使用前，称取315mg溶菌酶，加入EL Buffer，左右摇晃10-15次，大约30秒，直至溶液清亮，2～8℃保存。
2. 请在第一次使用前，向BA Buffer 中加入 21ml 乙醇(无水）并混合均匀。
3. 请在第一次使用前，向Wash Buffer 中加入63ml乙醇（无水）并混合均匀。
4. 为了保证最优化的提取效果，细菌细胞数最大不超过$5\times10^9$，OD600在1.0～2.0之间。
5. 如果RS Buffer 中出现沉淀或沉淀，请于56° C环境中适当温育，待浑浊物质消失后再使用。

## 操作步骤

1 收集细菌，取0.5-4ml细菌（最多$5\times10^9$个细菌），13,000 x g 离心一分钟，弃上清。尽可能吸净上清。
2 加入100μl EL Buffer，使用tip头吹打均匀。
3 37℃温育10-60分钟。
**注意：对于革兰氏阴性细菌，可将温浴时间缩短至10-15分钟，对革兰阳性细菌，可将温育时间延长至30-60分钟。**
4 加入100μl RS Buffer，随后分别加入10μl PK Solution，充分混匀。可选：加入2μl RNase A（20mg/ml）并混匀。
**注意：对于部分革兰氏阳性细菌和厌氧菌，请加入2μl RNase A（20mg/ml），混合充分将有助于裂解。**
5 于56℃环境中温浴15分钟，然后移出。对难于裂解的样本请适当延长温浴时间。
6 加 200μl GA Buffer 并混合均匀。
7 于 13,000 x g 离心1分钟。
8 将上清液转移到一个新的1.5ml离心管。
**注意：如果上清中出现无色粘稠物质，请全部转移到新离心管内。**
9 加 400μl 的 BA Buffer，并混合均匀。
10 将混合液体转移至Spin column。于13,000 x g离心1分钟， 并弃去接液管中液体。
11 向 Spin column中加入500μl的G Binding Buffer。于 10,000 x g 离心1分钟，并弃去接液管中液体。
12 向 Spin column 中加入500μl的Wash Buffer。于 10,000 x g 离心1分钟， 并

## 试剂盒组成 (100T)

| 成分 | 数量 |
|---|---|
| Proteinase K | 1ml |
| EL Buffer | 10ml |
| RS Buffer | 10ml |
| GA Buffer | 20ml |
| BA Buffer | 20ml (add 21ml ethanol before use) |
| G Binding Buffer | 50ml |
| Wash Buffer | 42ml (add 63ml ethanol before use) |
| Elution Buffer | 20 ml |
| Spin column | 100 |
| 说明书 | 1 份 |

## 储存条件

- 除 PK Solution 保存于 2-8℃外，其余保存于室温 （15-25℃）。
- 所有试剂可稳定保存 18 个月。

## 简介

本产品采用了一套基因组DNA提取试剂系统，提供了一套从各种细菌中提取基因组DNA的简单、快速、经济的方法,提取的细菌包括革兰阳性和革兰阴性细菌。 该方法是以选择性Biospin膜系统为基础，可以在1小时以内完成如基因组DNA这样的高分子量DNA的提取纯化。该方法的步骤简单，不需使用特殊的昂贵设备，而且完全避免了与一些有毒试剂的接触（如酚、氯仿等）。通常，使用本产品从$5\times10^9$个细菌中最多可以提取30μg的基因组DNA。

提取纯化后的 DNA，可以直接用于 PCR/Real time PCR， sequencing， Southern blot， mutant analysis，SNP 等下游应用实验。

## 原理

首先收集好的细菌在EL缓冲液中酶解，接着通过RS Buffer和PK Solution的裂解作用，基因组DNA被释放出来。在加入GA缓冲液并离心之后，杂质被去除。然后，在随后加入的BA Buffer 和G Binding Buffer中适当的盐分及pH值的作用下，DNA被特异吸附于Biospin膜上。通过洗涤，可将蛋白质等残留的杂质去除。最后使用Elution Buffer将DNA从膜上洗脱下来，从而获得基因组DNA。

## 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

## Important notes

1. Gram negative bacteria(optional step):Please add 315mg Lysozyme to EL Buffer,mix thoroughly for 30seconds or so until the solution is clear,stored at 2-8℃.
   Gram positive bacteria:Please add 315mg Lysozyme to EL Buffer,mix thoroughly for 30seconds or so until the solution is clear,stored at 2-8℃.
2. Please add 21ml absolute ethanol to BA Buffer and mix thoroughly before use.
3. Please add 63ml absolute ethanol to Wash Buffer and mix thoroughly before use.
4. In order to optimize the effective result, the appropriate number of bacterial cells is at most $5\times10^9$, which OD600 is between 1.0~2.0.
5. RS Buffer may form precipitates upon storage. If a precipitate has formed, incubate the buffer at 56°C until the precipitate has fully dissolved.

## Protocol

1. Harvest 0.5～4.0ml cells (maximum 2 x $10^9$ cells) in a micro centrifuge tube by centrifuging for 1 min at maximum speed. Discard supernatant as far as possible.
2. Resuspend pellet in 100 μl EL Buffer. Mix the mixture thoroughly with tip.
3. Incubate at 37℃ for10-60 minutes.
   **For gram negative bacteria ,Incubate at 37℃ for 10-15minutes.For gram positive bacteria,Incubate at 37℃ for 30-60mins or more times.**
4. Add 100μl RS Buffer, ,10μl PK Solution, respectively, mix thoroughly.
   Optional: add 2μl RNase A(20mg/ml) and mix thoroughly.
5. Incubate at 56℃ for 15 minutes. And remove the tube from 56℃.If the sample is difficult to be lysised,please extend the incubation time。
6. Add 200μl GA Buffer and mix thoroughly.
7. Centrifuge at 13,000 x g for 1 minutes.
8. Transfer the supernatant to a new 1.5ml tube. If some mucous material exist in the supernatant,please transfer them to the new 1.5ml tube.
9. Add 400μl BA Buffer. Mix thoroughly.
10. Transfer the mixture to the spin column. Centrifuge at 13,000 x g for 1 minute. Discard flow-through.
11. Add 500μl of the G Binding Buffer into the spin column. Centrifuge at 10,000 x g for 1

minute. Discard flow-through.

12. Add 500µl Washing Buffer to the spin column. Centrifuge at 10,000 x *g* for 1 minute. Discard flow-through.
13. Repeat step 12th.
14. Centrifuge for an additional 1 minute at 10,000 x *g* and transfer the spin column to a sterile 1.5ml micro centrifuge tube.
15. Add 100µl Elution Buffer, incubate at room temperature for 1 minute.
16. Centrifuge at 10,000 x *g* for 1 minute. Remove Spin column, the buffer in the micro centrifuge tube contains the DNA.
17. The purified DNA can be used directly for kinds of downstream molecular biological experiments. Store at -20℃ if not used immediately.

# FAQ

**1. Which kind of bacteria we can extract genomic DNA from?**

A We can extract genomic DNA from a majority of bacteria, including Gram-Negative Bacterial and Gram-Positive. We can break up the construct of bacteria's cell.

**2. What can we do if RS buffer solution turn to muddiness ?**

A Please shake up thoroughly.

**3. Is RNase A necessary for the test?**

A Determined by test aim. It's not necessary, if the extracted genomic DNA is used to backward test of molecular biology such as PCR, enzyme digestion and so on; It's necessary for RNA digesting adequately, if we want to get high purity genomic DNA.

**4. Is there organic solution in the extraction process?**

A No.

**5. How long about the extracted bacteria genomic DNA fragments?**

A Among 20-150KB.

**6. What's the matter about the small quantity of the extracted genomic DNA?**

A Pay attention to the following items

a) Please check that the bacteria is in the position of logarithmic phase when the bacteria reproduce fast.

b) Check to add lysozyme into EL buffer solution and shake up sufficient.

c) After add EL buffer solution, break up the bacteria mass at the bottom of tube and provide enough incubate time at 37℃.

d) Ensure that other extraction processes follow with the specification strictly.

7. **What's the quantity range of the bacteria for extraction?**

A Usually, the bacteria's quantity is among $10^7$~$10^8$/ml, which was incubated on liquid culture medium and was in the position of logarithmic phase, and the max. quantity is $5\times10^9$.Please take count of cells if the bacteria was incubated on solid culture medium. Pay attention to enrichment when the bacteria's quantity is small.

# Analysis DNA

⊕**Absorbance anlysis**

Get some DNA，dilute it a advisable factor with elution buffer. Survey the OD260 ，OD280 and OD320 .

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

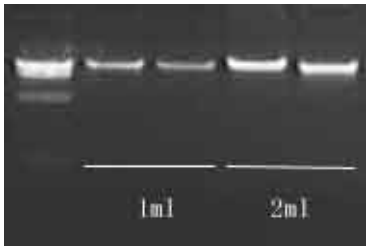
target：2.0≥$OD_{260-320}$/ $OD_{280-320}$≥1.7

Notice: 1.0≥$OD_{260-320}$≥0.1, the result of ratio is much reliable.

⊕**Agarose Gel Analysis**

**0.8～1% Agarose gel**

Example 1：different amount




Example 2：G+: gram-negative, G-: gram-positive