Biospin Gel Extraction Kit

Cat# BSC02M1

TECHNICAL SUPPORT:

For technical support, please dial phone number ：
0086-571-87774567-5278 or 5211, fax to 0086-571-87774303
email to reagent@bioer.com.cn.

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Kit Components (100T)

Component	Amount
Extraction Buffer	170ml
Wash Buffer	10ml×2
Elution Buffer	20ml
Spin columns	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

Biospin Gel Extraction Kit provides a simple, rapid and effective method for purification of DNA fragments from agarose gel in TAE or TBE buffer. DNA fragments ranging from 60bp to 23kb are purified from up to 3% standard or high/low-melt gel using Spin column. Purified DNA can be used directly for kinds of downstream molecular biological experiments such as cloning, sequencing, restriction enzyme digestion and so on.

Technical Information

Method	Work time	Column volume	DNA size range	Elution recovery	Sample volume
Spin column	16 min for 2 samples	750µl	60bp～23kb	≥99%	Up to 400mg gel slice

Agerose type	Electrophoresis buffer	Incubate temperature
High/low melt agarose	TAE/TBE buffer	50℃（low-melt agarose） 55℃（standard agarose）

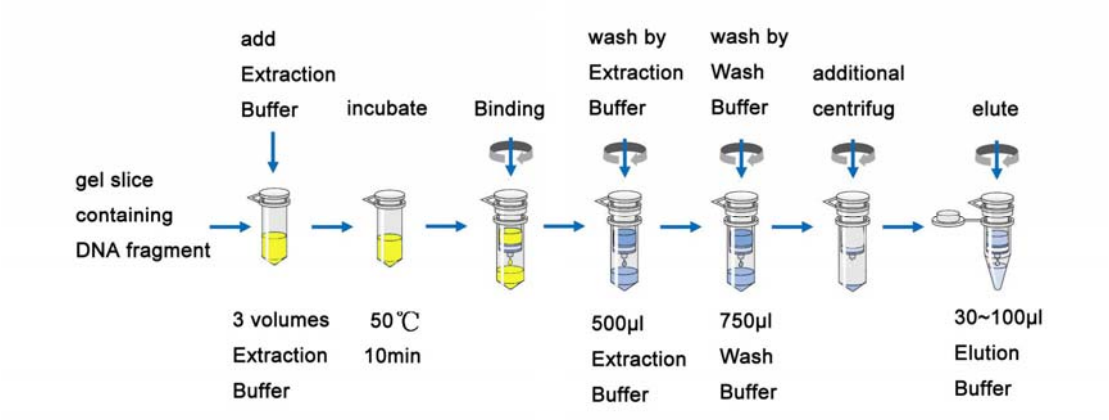
Apparatus and Materials to Be Supplied by the User

* sterile 1.5 and 2.0 ml microcentrifuge tubes * 10µl/100µl/1000µl tips * Absolute ethanol * isopropanol *3 M sodium acetate(pH 5.0), may be necessary * microcentrifuge capable of 14,000g * Vortex mixer

Preparation

1. The yellow color of Extraction Buffer indicates pH ≤ 7.8.
2. Add 40ml ethanol (as the volume be marked on bottle label) to Wash Buffer and mix well.
3. Close the lid after using the Extraction Buffer as soon as possible.
4. The fitting elution volume is 50µl, user can adjust its volume if necessary.
5. 3 M sodium acetate(pH 5.0), may be necessary.

Procedure



1. **Excise the DNA fragment from the agarose gel whith a clean, sharp scalpel.**
   Minimize the size of the gel slice by removing extra agarose.
2. **Weigh the gel slice and add 3 volumes of Extraction Buffer to 1 volume of gel slice (100mg＝100µl).**
   For example, add 300µl Extraction Buffer to each 100mg of gel. The gel slice should not more than 400mg per test.
3. **Incubate at 50℃ until the gel melts in a heating block and vortex the tube every 2-3 minutes during the incubation.**
   Usually, It is 10 min. If the color of the mixture is purple add 10ul of 3 M sodium acetate(pH5.0),and mix. The color will return to yellow.
4. **Optional: Add 1 volume of isopropanol to 1 volume of gel and mix.**
   No need to add isopropanol in the case the fragments ＞500bp and ＜4 kb .
5. **Apply the sample to Spin column, centrifuge for 1 min at 6,000×*g*. Discard the flow-through.**
   If the sample volume is more than 750µl, simply load and spin again.
6. **Add 500µl Extraction Buffer to Spin column, centrifuge for 30~60s at 12,000×*g*. Discard the flow-through.**
7. **Add 750µl Wash Buffer to Spin column, centrifuge for 30~60s at 12,000×*g*.**

Discard the flow-through.

If the DNA will be used for salt sensitive applications, let the spin column stand 2~5 min after addition of Wash Buffer, before centrifuging.

8. **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.

9. **Add 30~100μl Elution Buffer, H_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. But not less than 20μl.

10. **Centrifuge for 1 minute at 12,000×*g*. The buffer in the microcentrifuge tube contains the DNA.**

    The extracted 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 melted (binding mixture turns purple), it will leads to inefficient DNA binding. Please add 0.1volume 3M sodium acetate（pH5.0）to the sample and mix. The color of the mixture will turn yellow. If the binding mixtures with color changes to purple.
2. The electrophoresis buffer has been repeatedly used ，it will leads to low recovery. Please use new electrophoresis buffer.
3. Incubate the Elution Buffer in 30～60℃， it will increase the yields.

Absorbance problem

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

Recovery counting

1. Since the pre-extracting samples usually contains non-targeted DNA fragments, primer , dNTP and so on, the recovery can't be counted according to the absorbance.

2. Do the Electrophoresis ， use the DNA fragments pre-extracting together. Use gel imaging system to take photos, and then do contrast analysis on electrophoresis analysis software.

Note: Elecrophoresis conditions and photo-shoot conditions resulted produce a lot of effects to the final result, please operate carefully.

Analysis DNA

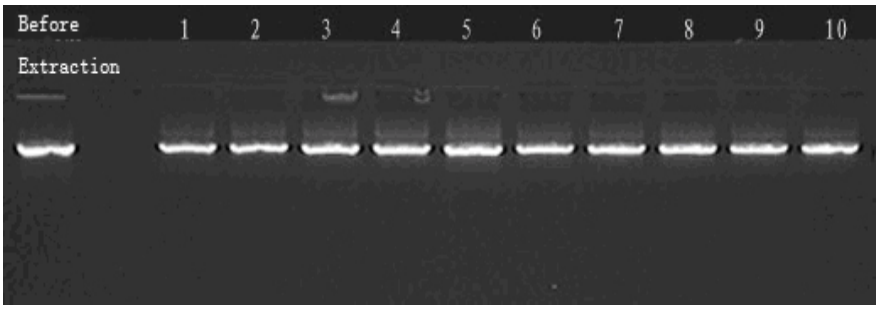
⊕ Absorbance anlysis

Get some DNA, diluted in a advisable factor with elution buffer.
Survey the $OD_{260,}$ OD_{280} and OD_{320}.
Expressions: concentration（μg/ml）＝$50\times OD_{260}\times$dilution fact
Notice: $1.0\geq OD_{260}\geq 0.1$, the result of ratio is most reliable.

⊕ Agarose Gel Analysis:

0.8～1％ Agarose gel

Example 1：



Example 2: Elution Volume versus DNA Yield