Biospin Gel and PCR Purification Kit

Cat# BSC02M1A

TECHNICAL SUPPORT:
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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 and PCR Purification Kit provides a simple, rapid and effective method for purification of DNA fragments from agarose gel in TAE or TBE buffer 、 PCR or enzymatic reaction. 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

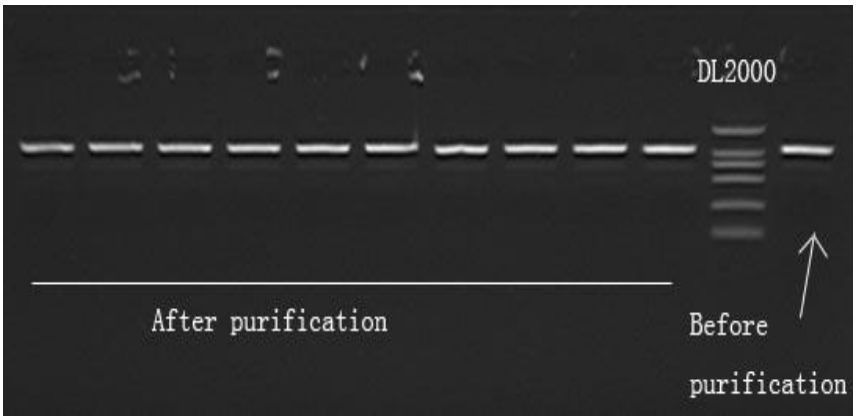
Method	Work time	Column volume	DNA size range	Elution recovery	Sample volume
Spin column	16 min for 2 samples	750μl	60bp～23kb	≥99%	Up to 400mg gel slice

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

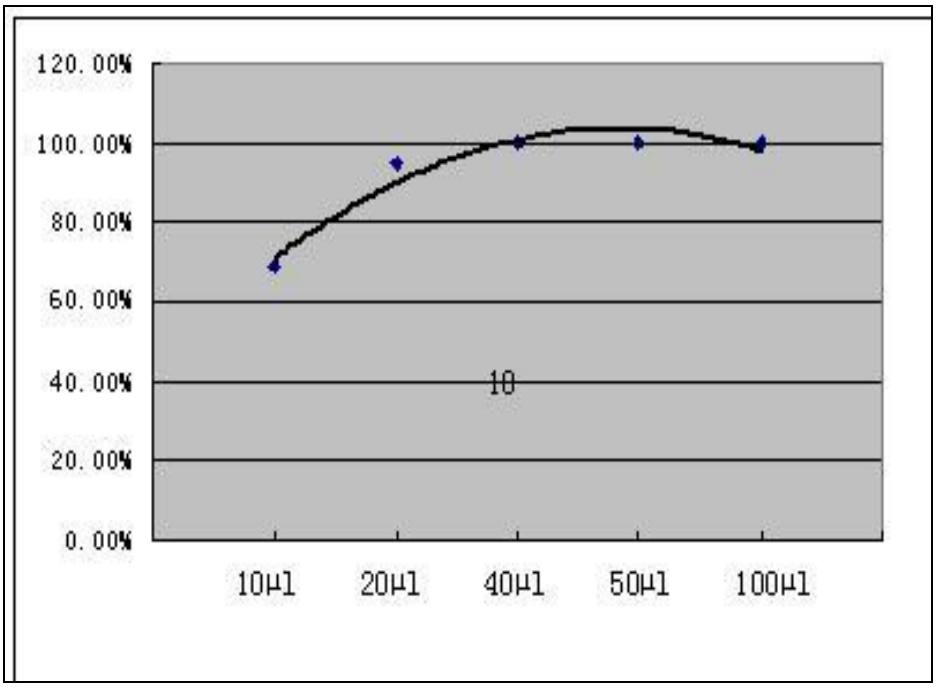
Apparatus and Materials to Be Supplied by the User

* 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

PCR purification example 2：



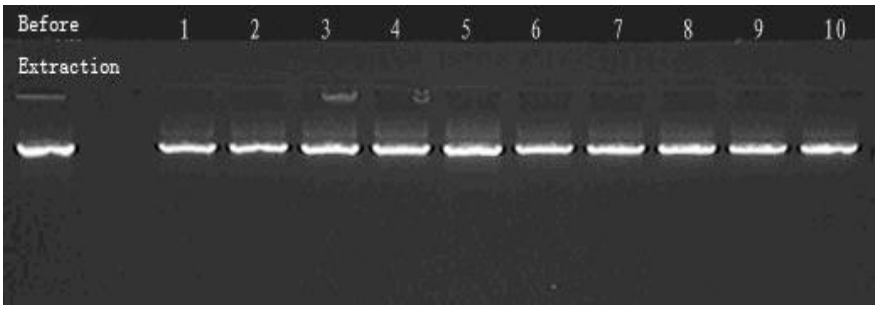
PCR purification example3：Elution Volume versus DNA Yield



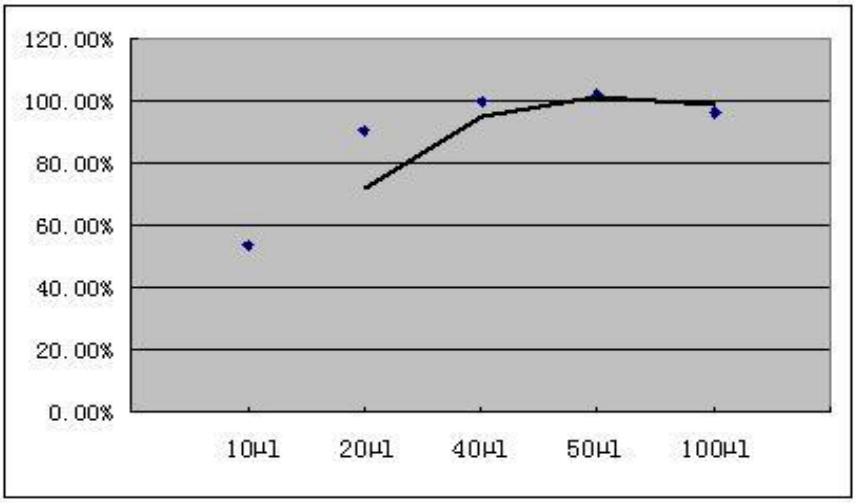
⊕**Agarose Gel Analysis:**

0.8～1％ Agarose gel

Gel purification example 1：



Gel purification example 2: Elution Volume versus DNA Yield



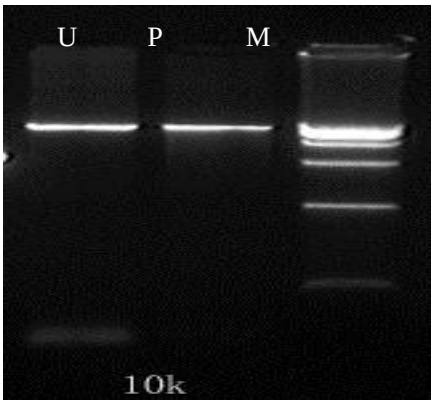
PCR purification example 1：

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

U: unpurified P: purified M: Marker

Target DNA fragment→

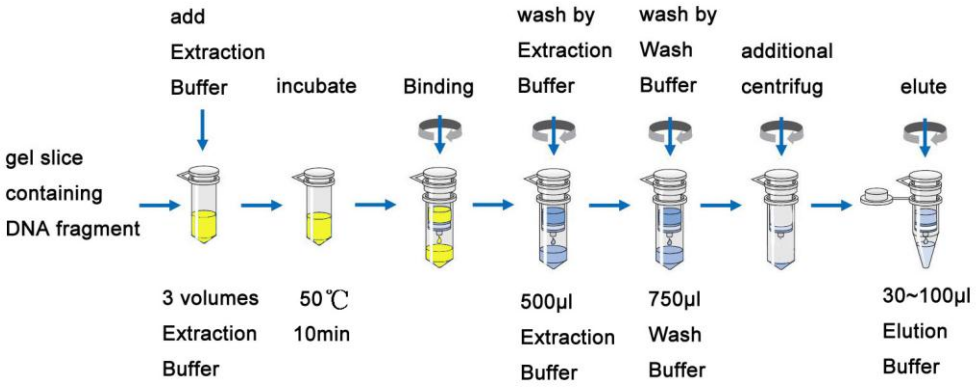
Primer→

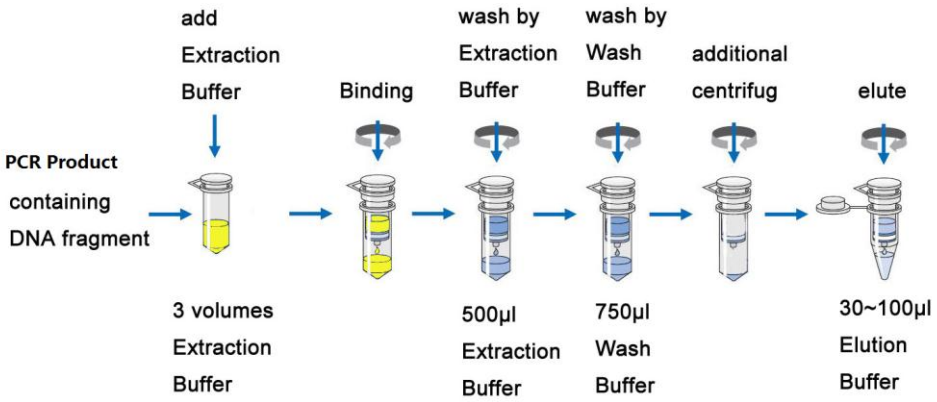


Preparation

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





Gel purification pre-treatment

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.

PCR purification pre-treatment

1. **Transfer the PCR or enzymatic reaction product to 1.5ml microcentrifuge tubes.**
2. **Add 3 volumes of Extraction Buffer to 1 volume of the PCR or enzymatic reaction product and vortex.**
   The maximal volume added every time should not exceed 200μl .

Binding、Wash and Elution

1. **Optional: Add 1 volume of isopropanol to 1 volume of gel or PCR and mix.**
   No need to add isopropanol in the case the fragments ＞500bp and ＜4 kb .
2. **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.
3. **Add 500μl Extraction Buffer to Spin column, centrifuge for 30~60s at 12,000×*g*. Discard the flow-through.**
4. **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.
5. **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.
6. **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.
7. **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.
3. Pay close attention to operation to reduce error, for electrophoresis and photo condition will affect comparison result.

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

The size of DNA and yield

This kit is available for DNA whose size is larger than 60bp, 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）＝$50 \times OD_{260} \times$dilution fact

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