QuickClean II Gel Extraction Kit **Cat. No. L00418**
Technical Manual No. 0594 **Version: 05232014**

I DESCRIPTION

QuickClean II Gel Extraction Kit offers a simple, rapid and effective method to extract and purify DNA from agarose gel in TAE or TBE buffers. This kit is designed to increase the recovery of DNA fragments from 60 bp to 23 kb DNA fragments which are purified from up to 3% standard or high/low-melt agarose using spin column. The extracted DNA can be used directly for kinds of downstream molecular biological experiments such as cloning, sequencing, restriction enzyme digestion and so on.

II KEY FEATURES

- **Easy to Perform**: Simple procedure to extract and purify DNA up to 23 kb from standard or high/low-melt agarose gels in TAE or TBE buffers.
- **Fast Preparation Time**: Complete each QuickClean's simple and rapid procedure in 8 minutes.
- **High Binding Capacity**: The maximum amount of the sample per spin column is 400 mg gel slice. The recovery rates are up to 90% for the 100 bp – 3 kb DNA fragments size range.
- **High Purity**: The kit completely removes nonnucleic acid contaminants and inhibitors, such as agarose, proteins, salts, and ethidium bromide during washing process.

III COMPONENTS

The components of QuickClean II Gel Extraction Kit are described in the table below.

Components	Volume (50 rxns)	Volume (100 rxns)
Binding Buffer II	85 ml	170 ml
Wash Buffer	10 ml	10 ml × 2
Elution Buffer	10 ml	20 ml
Spin columns	50 (column volume: 750 µl)	100 (column volume: 750 µl)

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IV APPLICATIONS

QuickClean II Gel Extraction Kit is suitable for the following applications:

- Restriction enzyme digestion
- Transformation
- PCR and cloning
- Sequencing
- *In vitro* transcription

V STORAGE

The kit should be shipped and stored dry at room temperature (15-25°C). So stored, the kit is stable for up to 18 months.

VI PROTOCOL

Materials Supplied by the User

You need to prepare the reagents and equipments as following for gel purification:

- 1X TAE/TBE buffer
- Sterile microcentrifuge tubes (Volume: 1.5 ml and 2.0 ml)
- Sterile tips (Volume: 10 µl/100 µl/1000 µl)
- Absolute (100%) ethanol
- Isopropanol
- 3M sodium acetate (pH 5.0), may be necessary
- Microcentrifuge (capable of 14,000×g)

Reagents Preparation

Please note that the following solutions should be stored at room temperature.

1. The pH value of the Binding Buffer II should be at pH 7.8 or lower in process before binding.
2. Add 40 ml ethanol (as the volume be marked on bottle label) to Wash Buffer and mix well.
3. After using the Binding Buffer II, close the lid as soon as possible.
4. The optimum elution volume is 50 µl, users can adjust its volume if necessary.
5. 3 M sodium acetate (pH 5.0), may be necessary.

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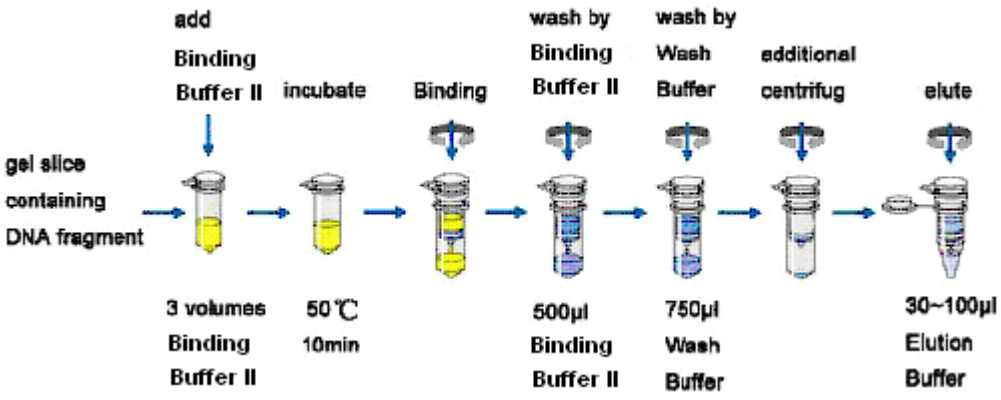
Test Procedure



1. Excise the DNA band from the agarose gel with a clean, sharp scalpel.

Minimize the size of the gel slice by removing extra agarose.

2. Weigh the gel slice.

Then Add 3 volumes of Binding Buffer II to 1 volume of gel slice (100mg≈100ul),the gel slice should not be more than 400mg per test)

3. Incubate at 55 °C for 10 minutes with occasional vortexing or until the gel slice has been completely dissolved. (Note: 50°C is used for low-melt agarose).

Usually the color of the mixture will be yellow. If the color of the mixture is purple, add 10 μl of 3 M sodium acetate (pH 5.0) and mix well. 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 of the DNA fragments from 500 bp to 4 kb

5. Transfer the sample to Spin column, centrifuge at 6,000×*g* for 1 min. Discard the flow through until the sample is processed completely.

If the sample volume is more than 750 μl, load and centrifuge again using the same column.

6. Add 500 μl Binding Buffer II to Spin column, centrifuge at 12,000 ×*g* for 30~60s. Discard all flow through liquid.

7. Add 750 μl Wash Buffer to Spin column, centrifuge at 12,000 ×*g* for 30~60s. Discard all flow through liquid.

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

8. Centrifuge at 12,000 ×*g* for an additional 1 minute 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 30~100 μl Elution Buffer, ddH_2O or TE Buffer to the Spin column and let it stand for 1 minute at room temperature.

DNA pellet was not sufficiently washed by using less than 20 μl of Elution Buffer. Adjust the volume of elution buffer according to needs if necessary.

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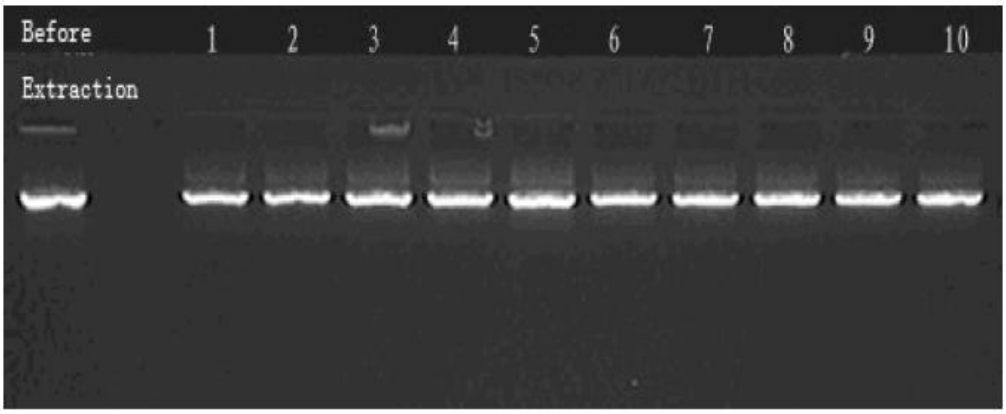
10. Centrifuge at 12,000 ×*g* for 1 minute. The buffer in the micro-centrifuge tube contains the DNA.
The extracted DNA can be stored at -20 °C if not used immediately.

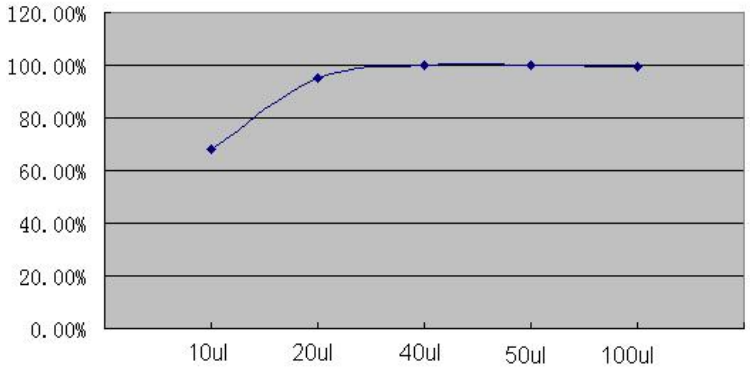
VII EXAMPLES USING THE KIT

Figure 1. The following example shows the DNA recovery after extracting 1007 bp PCR products using this kit. The DNA yield is up to 95% according to the contrast of the gray scale.



Lane 1~10: 1007 bp PCR products after extraction.

Figure 2: The following example show DNA yield performance according to the Elution Volume change .The elution volume will affect the DNA yield if less than 20 µl.



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VIII Troubleshooting

Problem	Recommend
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 marked on bottle label.
Low recovery	1. The Binding Buffer II 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 (pH 5.0) to the sample and mix if the binding mixtures with color changes to purple. The color of the mixture will turn yellow. 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**°C**, it will increase the yields.
Absorbance	Absorbance is the difference from sample to criterion, please use the Elution Buffer to adjust zero value and dilute the sample.
Recovery counting	Since the pre-extracting samples usually contain non-targeted DNA fragments, primer, dNTPs and so on, the recovery can't be counted according to the absorbance.

IX Ordering Information

QuickClean II Gel Extraction Kit Cat. No. L00418-50 Cat. No. L00418-100

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