QuickClean II PCR Extraction Kit

Cat. No.: L00419 Version 2018-07-16

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I Description

The QuickClean II PCR Extraction Kit provides a simple, rapid and effective method for purification of 60 bp to 10 kb DNA fragments from PCR and other enzymatic reactions. 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.

II Key Features

- **Easy to Perform:** Complete procedure to purify DNA from two samples in 6 min.
- **High Capacity:** Up to 20 µg DNA each column
- **High Purity:** The kit completely removes non-nucleic acid contaminants and inhibitors, such as agarose, proteins, salts, and ethidium bromide are removed during washing process.

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III Contents

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

Kit Contents	Volume (50 rxns)	Volume (100 rxns)
Binding Buffer	10 mL	20 mL
Wash Buffer	15 mL	15 mL × 2
Elution Buffer	10 mL	20 mL
Spin columns	50 (column volume: 750 µL)	100 (column volume: 750 µL)

IV Application

The QuickClean II PCR Extraction Kit enables the fast purification of high-quality DNA products from PCR reactions and other enzymatic reactions. The extracted DNA is immediately ready for further downstream applications such as the following:

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

V Storage

The kit is shipped and stored at room temperature (15 25°C). The kit can be stored for up to 18 months if all components are kept at room temperature.

VI Protocol

Materials Supplied by the User

You will need reagents and equipment for PCR purification:

- ◇ Sterile microcentrifuge tubes (Volume: 1.5 mL)
- ◇ Sterile tips (Volume: 10 µL/100 µL/1000 µL)

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- ◇ Absolute (100%) ethanol
- ◇ Microcentrifuge (capable of 14,000× g)

Important notes

Please read the following notes before the PCR Purification procedures.

1. Add ethanol (100%) (The volume of ethanol to be added is also shown on the bottle labels) to Wash Buffer and mix well before using
2. Close the lid after using the Binding Buffer as soon as possible.
3. The optimum volume is 50 μL for Elution Buffer, user can adjust its volume if necessary.

Procedure

1. Transfer the PCR or enzymatic reaction product to 1.5 mL microcentrifuge tubes.
2. Add 2 volumes of Binding Buffer to 1 volume of the PCR or enzymatic reaction product and gently mix well. Note: Ensure that the maximum binding buffer input every time does not exceed 200 μL. 3 Apply all above mixture to the Spin column by decanting or pipetting. And centrifuge for only 1 min at 6,000×g.
3. Discard all flow-through and place the column back in the same tube.
4. Wash the Spin column by 650 μL Wash Buffer in centrifuging for 30-60 s at 12,000×g. Discard the flow-through liquid and repeat Step 5 again.
5. Centrifuge for an additional 1 min at 12,000×g and transfer the Spin column to a sterile 1.5 mL microcentrifuge tube. By this step, there will be no residual liquid in the column
6. Add 50 μL Elution Buffer, ddH2O or TE Buffer to the center of the Spin column and let it stand for 1 min at room temperature. Then centrifuge for 1 min at 12,000×g. The volume of Elution Buffer should be adjusted if necessary.
7. Store the micro-centrifuge tube containing purified plasmid DNA at -20°C if not use immediately.

VII Examples

1 Absorbance analysis

Get some DNA, diluted in an 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≥$OD_{260-320}$/ $OD_{280-320}$≥1.8
Notice: 1.0≥OD_{260}≥0.1, the result of ratio is much reliable.

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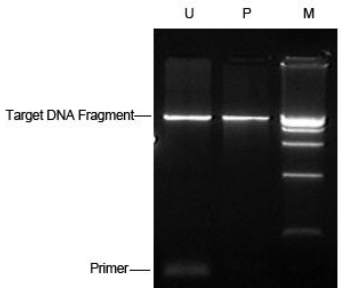
2 Agarose Analysis

Figure 1: The example as below shows performance after impurity is removed from target DNA fragment (10 kb) using our kit according to the contrast of the gray scale.



Lane U: unpurified PCR products (10 kb)
Lane P: purified PCR products
M: DL15,000 DNA Marker (Takara)

Figure 2: According to the absorbance shown is a comparison of the absorbance result before and after PCR products (1007 bp) extraction using QuickClean II PCR Extraction Kit, the DNA yield is 96% according to the contrast of gray scale.

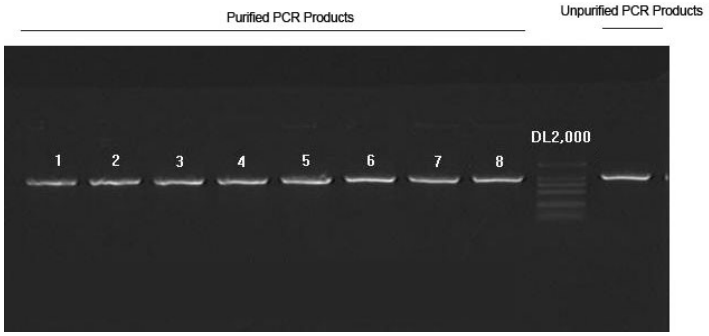


Figure 3: Elution Volume versus high DNA Yield during purification of 10 kb target DNA fragments.

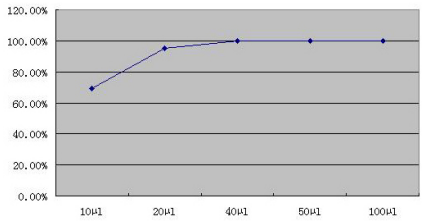


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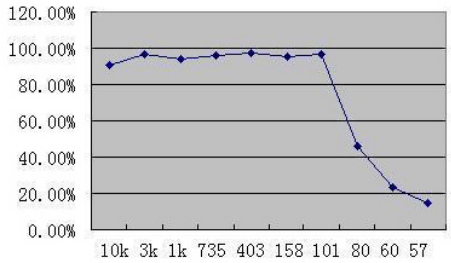
Figure 4: Recovery of DNA is up to 97% for purification 100 bp to 10 kb DNA fragment from primers, nucleotides, polymerases, and the purification yield is up to 95% for purifying 100 bp or lower size DNA fragments .



VIII Trouble Shooting

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 be marked on bottle label.
Low recovery	1. The extraction buffer is acidic buffer, if the pH increases after gel extracted, it will leads to inefficient DNA binding. Please add 0.1volume 3M sodium acetate (pH 5.0). 2. Incubate the Elution Buffer in 30-60 °C, 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.
Low or no yield	1. Because there are usually non-target 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 50 bp, thus to remove primer of PCR product efficiently.

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IX Ordering Information

Product Name	Cat. No.
QuickClean II PCR Extraction Kit	L00419
High-Stability PCR Kit	L00342
Green *Taq* DNA Polymerase, 100 µl (500 U)	E00043
10X *Taq* Buffer (with Mg2+), 1.5 ml	B0005
Stabilized dNTP Mix, 300 µl (10 mM each)	C01689

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