Kit Components(100T)

Component	Amount
Binding Buffer	20ml
Wash Buffer	30ml
Elution Buffer	20ml
Spin column	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

The Kit provides a simple, rapid and effective method for purification of DNA fragments from PCR or enzymatic reaction. DNA fragments ranging from 60bp to 10kb can be purified. The yield of DNA with size lower than 100bp is 23~95%, while the yield of DNA with size from 0.1kb to 10kb is 90~97%. 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.

Technical Information

Method	Work time	Column volume	DNA size range	Elution recovery	Sample volume
Spin column	6 min for 2 samples	750μl	60bp ~ 10kb	≥99%	Up to 100 μl reaction

Apparatus and Materials to Be Supplied by the User

* sterile 1.5 microcentrifuge tubes * 10μl/100μl/100 0μl tips
* microcentrifuge capable of 14,000g * Vortex mixer * Absolute ethanol

Important notes

1. Add ethanol (as the volume be marked on bottle label) to Wash Buffer and mix well
2. Close the lid after using the Binding Buffer as soon as possible.
3. The suitable volume is 50ul for Elution Buffer, user can adjust its volume if necessary.

Procedure

1. **Transfer the PCR or enzymatic reaction product to 1.5ml microcentrifuge tubes.**
2. **Add 2 volumes of Binding Buffer to 1 volume of the PCR or enzymatic reaction product and vortex.**

   The maximal volume added every time should not exceed 200μl .
3. **Apply the mixture to the Spin column by decanting or pipetting.**
4. **and centrifuge for 1min at 6,000×*g* . Discard the flow-through.**
5. **Wash the Spin column by 650μl Wash Buffer in centrifuging f or 30-60 seconds at 12,000×*g* . Discard the flow-through.**
6. **Repeat Step 5th once.**
7. **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
8. **Add 50μl Elution Buffer , ddH2O 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.
9. **Centrifuge for 1 minute at 12,000×g. The buffer in the microcentrifuge tube contains the DNA.**
10. **The purified plasmid 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 excated, it will leads to inefficient DNA binding. Pleas add 0.1volume 3M sodium acetate（pH5.0）.

2） Incubate the Elution Buffer in 30～60℃ ， 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.

How to calculate yield

1) Because there are usually nonpurpose 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 50bp, 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×OD_{260}×dilution fact

target：$2.0 \geq OD_{260-320} / OD_{280-320} \geq 1.8$

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

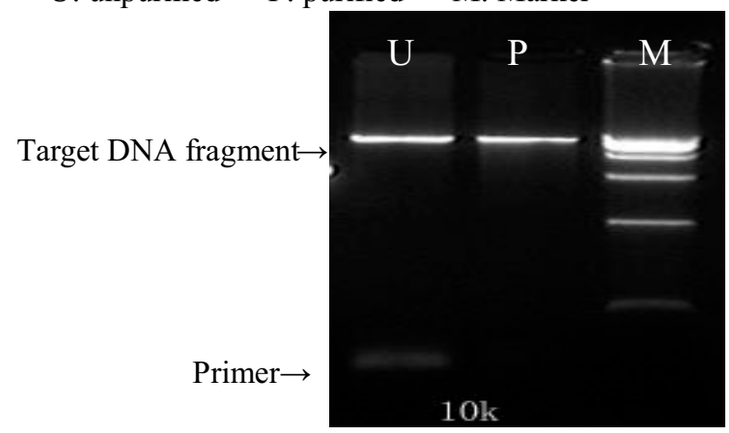
⊕ **Agarose Gel Analysis**

0.8～1% Agarose gel

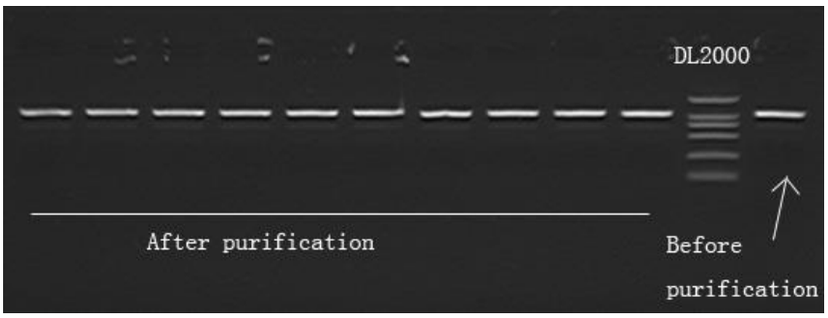
Example 1：

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

U: unpurified P: purified M: Marker



Example 2：



Example3：Elution Volume versus DNA Yield