Dokdo-Prep™
PCR Purification kit

User Manual

Cat no. EBD-1004

Storage Conditions : Room Temperature
For Research Use Only

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Overview

The Dokdo-Prep™ PCR Purification Kit is a convenient system for fast and reliable purification of double- or single-stranded PCR products. The method uses Dokdo-Prep™ PCR purification technology to recover DNA bands 100 bp - 10 kb free from oligonucleotides, nucleotides, and polymerase in yields exceeding 90 to 95%. Binding conditions are adjusted by addition of a specially formulated buffers, and the sample is simply applied to spin column after mixing PCR products with Binding Buffer. Purified DNA fragment is ready for restriction enzyme digestion, labeling, ligation, transformation, and sequencing reactions.

Kit Contents

Components	Amount	Storage
Dokdo-Prep™ Column *	200 ea	Room Temperature
PCR Binding Buffer	100 ml	Room Temperature
Wash Buffer**	20 ml x 2	Room Temperature
Elution Buffer	15 ml	Room Temperature
Manual	1 ea	-

* Dokdo-Prep™ Column is contained 2 ml collection tube

** **Before use, add 80 ml of absolute ethanol (>98%) to 20 ml Wash Buffer.**
For other volumes of wash buffer, simply add enough ethanol to make a 4 : 1 ratio (Ethanol volume : Wash Buffer volume).

Quality Control

The performance of Dokdo-Prep™ PCR Purification Kit is monitored routinely on a lot number. This kit is tested by isolation of PCR products of 200 bp, 500 bp, and 1,500 bp from PCR reactions. The quality of the isolated DNA is checked by spectrophotometry, and agarose gel electrophoresis.

Centrifugation notes

All centrifugation steps are carried out at maximum speed (≥ 10,000g or ~13,000 rpm) in a conventional, table-top microcentrifuge.

Protocols

The Dokdo-Prep™ PCR Purification Kit is a convenient system to purify double-stranded DNA fragments from PCR reactions resulting in high end-concentrations of DNA. DNA Fragments ranging from 100 bp to 10 kb are purified from oligonucleotides primer, nucleotides, polymerase, and salts using spin columns in a microcentrifuge.

1. For each completed PCR amplification, transfer the aqueous phase to a clean 1.5 ml microcentrifuge tube.

2. Add 250 μl of PCR Binding Buffer to each 50 μl of PCR reaction mix (not including oil) and mix by tapping. (PCR Binding Buffer : PCR mix = 5 : 1)
If there is any visible precipitates in PCR Binding Buffer, completely dissolve at 37℃ before use

3. To bind DNA on filter membrane, apply the mixture to the spin column and centrifuge 13,000 rpm for 1 min at room temperature.
For maximum recovery, transfer all traces of sample to the column.

4. Discard the flow-through and reinsert the column into the collection tube.

5. To wash, add 750 μl Wash Buffer to the column and centrifuge 13,000 rpm for 1 min.

6. Centrifuge for 1 min at maximum speed (> 13,000 rpm) to remove residual Wash Buffer completely.

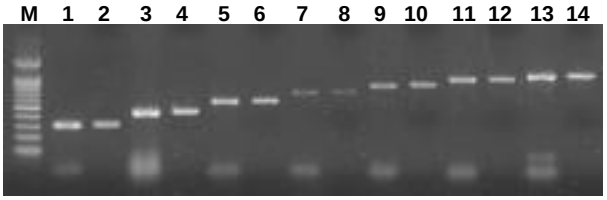
7. Transfer the column into a clean 1.5 ml microcentrifuge tube.

8. To elute DNA, add 50 μl of Elution Buffer or sterile distilled H_2O onto the column.
Alternatively, to increase DNA concentration, add 20 μl elution buffer to the center of the spin column.

9. Wait for 1 min and centrifuge for 1 min at maximum speed.
Store purified DNA at -20℃ or below for long-tern storage.

Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA. The average eluate volume is 18 μl from 20 μl loaded elution buffer volume. Elution efficiency can be changed by alteration in pH of elution buffer. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20℃ because DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

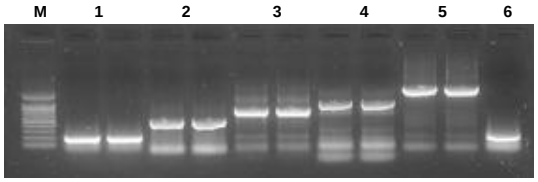
Fig.1. Premix PCR product purification



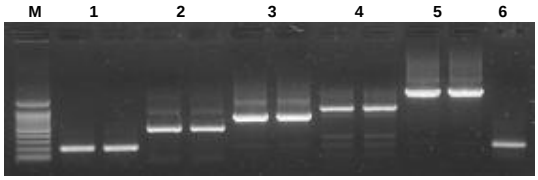
Analyzed on a 1% agarose
M : 100bp DNA ladder marker (EBM-1001)
Lane 1, 3, 5, 7, 9, 11, 13 Before purification
2, 4, 6, 8, 10, 12, 14 After purification
(309 bp, 444 bp, 537 bp, 699 bp, 837 bp, 975 bp, 1104 bp)

Fig.2. PCR product purification

Before purification



After purification



Analyzed on a 1% agarose
M : 100bp DNA ladder marker (EBM-1001)
Lane 1. 200 bp PCR DNA
2. 500 bp PCR DNA
3. 800 bp PCR DNA
4. 1 kbp PCR DNA
5. 2 kbp PCR DNA
6. 200 bp PCR DNA -premix PCR product

Customer & Technical Services

For technical assistance and more information please call one of the Elpis-Biotech., Inc.

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