Dokdo-Prep™
Gel Extraction Kit (spin-type)

User Manual

Cat no. EBD-1005

Storage Conditions : Room Temperature
For Research Use Only

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Overview

The Dokdo-Prep™ Gel Extraction Kit (spin-type) is suitable for rapid purification of DNA fragments after standard agarose gel electrophoresis. The kit uses spin column containing silica membrane to purify the DNA fragment. This enables simple extraction of DNA fragments from both TAE and TBE buffer-containing agarose gels without additional solutions or modifications to the protocol. DNA fragments can be also extracted from low-melting-point agarose gel.

- ▷ DNA fragments in the size range of 100 bp to 10 kbp can be extracted from gels.
- ▷ The procedure is brief, allowing completion of the protocol in 15 to 20 min.
- ▷ The recovery of DNA is 70～80%.
- ▷ Purified DNA is suitable for a variety of applications, including fluorescent DNA sequencing, transformation, restriction mapping, cloning, and labeling.

Kit Contents

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

* Dokdo-Prep™ Column contains 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).

Additional Materials Required

Absolute ethanol (>98%)
50～55 ℃ water bath or heat block

Centrifugation notes

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

Protocols

1. Carefully excise the gel slice containing DNA fragment from the agarose gel.
Place gel slice into clean 1.5 ml microcentrifuge tube.
Minimize the size of the gel slice as possible by removing excess agarose.
(< 250 mg agarose / tube).

2. Add 300 μl of Gel Extraction Buffer per 100 mg of gel slice (Gel Extraction Buffer : Gel slice = 3 : 1)
If there is any visible precipitates in Gel Extraction Buffer, completely dissolve at 37℃ before use.

3. Incubate sample at 50～55℃ for 5～10 min.
Invert tube 2～3 times every 2 min until the gel slices are completely dissolved.

4. Apply the solution to the spin column and centrifuge 13,000 rpm for 1 min at room temperature.
If the total volume is more than 750 μl, repeat this step.

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

6. Add 750 μl of Wash Buffer to the column and centrifuge for 1 min at maximum speed.
Be sure ethanol has been added to Wash Buffer before use.

7. Centrifuge for 1 min at maximum speed (> 13,000 rpm) to remove residual Wash Buffer in column.
Residual ethanol from Wash Buffer may inhibit subsequent application reactions.

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

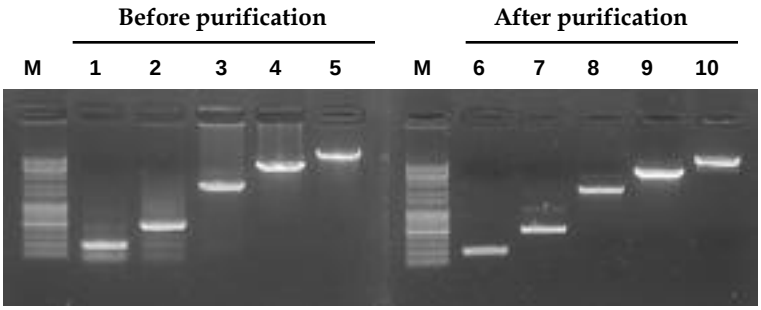
9. Add 30～50 μl of Elution Buffer or sterile distilled H_2O to the center of each column.

10. Wait for 1 min and centrifuge for 1 min at maximum speed.
Store purified DNA at -20℃ or below for long-term storage.
For fragments larger than 10 kb, resuspend the pellet by inverting and flicking the tube. Vortexing can cause shearing of large DNA fragments.

Basic principle

The Gel Extraction provides two simple techniques for separating DNA from agarose. Both techniques involve as a first step, the solubilization of agarose in the chaotropic salt, and the second step is either binding of the DNA to glass absorbent (glass binding method). DNA generally binds to silica in the presence of high concentration of chaotropic salt. Contaminants such as salts and soluble macromolecular components are removed by a simple washing step. DNA/silica binding is abolished when salt concentration is lowered. Since the DNA is eluted with either water or a low salt elution buffer, it can be used immediately in subsequent reactions without precipitation or other further manipulation.

Fig. 1. Analysis of DNA purification with Dokdo-Prep™ Gel Extraction Kit



Analyzed on a 1% agarose
M : 1Kbp plus 100bp DNA ladder marker (EBM-1003)
Lane 1, 6. 200 bp PCR DNA
2, 7. 500 bp PCR DNA
3, 8. 3 kbp BamH I cut DNA
4, 9. 5 kbp BamH I cut DNA
5, 10. 10 kbp BamH I cut DNA

Customer & Technical Services

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

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