Dokdo-Prep™ Bacterial Genomic DNA Purification Kit

User Manual

Cat no. EBD-1007

Storage Conditions : Room Temperature
For Research Use Only

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Overview

The Dokdo-Prep™ Bacterial Genomic DNA Purification Kit is designed for rapid isolation of highly qualified genomic DNA from various sample sources including Gram positive and Gram negative bacteria (for bacteria). It provides a fast, simple and inexpensive method requiring no expensive equipments and hazardous reagents. The Dokdo-Prep™ Genomic DNA Purification Kit uses advanced silica-based membrane technology for rapid and efficient purification of genomic DNA without organic extraction or ethanol precipitation. Purified DNA with this system is suitable for a variety of applications, including PCR amplification, membrane hybridization, and restriction.

Kit Contents

Components	Amount	Storage
Dokdo-Prep™ Column *	200 ea	Room Temperature
Cell Resuspension Buffer (G1)	100 ml	Room Temperature
gDNA Lysis Buffer ** (G2)	100 ml	Room Temperature
Wash Buffer +	20 ml x 2	Room Temperature
Elution Buffer	20 ml	Room Temperature
RNase A Solution	500 µl	4℃
Proteinase K ++	4 ml	4℃
Manual	1 ea	-

* Dokdo-Prep™ Column contains 2 ml collection tube

** gDNA Lysis Buffer is opaque at room temperature. However, if placed on 4℃, the buffer may be changed into transparent. This phenomenon don't influence on the efficiency of DNA preparation.

\+ **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).

++ Although Proteinase K is stable at 4℃, we recommend placing at -20℃ for longer storage.

Quality Control

The performance of Dokdo-Prep™ Bacterial Genomic DNA Purification Kit is monitored routinely on a lot number. The quality of isolated genomic DNA is checked by restriction digestion, agarose gel electrophoresis, and spectrophotometry by measuring absorbance at 230nm, 260nm, and 280nm.

Centrifugation Notes

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

Protocols

◆ Gram negative bacteria (ex, *E.coli* XL-1 Blue)

1. Pellet 1.5 ml of bacterial culture by centrifugation (8,000 rpm for 5 min) at 4℃.
   Wash twice with 500 µl of PBS or Cell Resuspension Buffer.

2. Add 200 µl of Cell Resuspension Buffer (G1) and mix well by vortexing.
   [optional] for RNase A treatment : Add 2 µl of RNase A (10 mg/ml) and wait for 5 min at room temperature.

3. Add 400 µl of gDNA Lysis Buffer (G2) and 20 µl of Proteinase K solution
   Then mix well by vortexing.

4. Incubate mixture for 15 min at 65℃.
   It is very important to lyse cells completely.
   To help lyse cells, mix by vortexing the tube every 2 min during the incubation.

5. Mix well by vortexing or pipetting.
   This step is semi-homogenization step to denature proteins. In addition, this step conduces to pass efficiently cell lysates through a column.

6. Transfer mixture on to the spin column.

7. Centrifuge for 1 min at 13,000 rpm.
   The cell lysates is very sticky at this step. If remaining solution is present in column after centrifugation, additional centrifugation is recommended to remove all traces of cell lysates.

8. Discard the flow-through.

9. Wash the column with 750 µl of Wash Buffer and centrifugation for 1 min at 13,000 rpm.

10. Discard the flow-through, and centrifuge the column for an additional 2 min at 13,000 rpm to completely remove residual Wash Buffer.

11. Place the column in a clean 1.5 ml microcentrifuge tube.

12. To elute DNA, add 50～100 µl of 55℃ pre-warmed Elution Buffer or H_2O on the center of spin column, wait for 1-5 min, and then centrifuge for 1 min at 13,000 rpm.

13. Store purified genomic DNA at -20℃ or below for longer storage.

If 230nm/260nm ratio of purified DNA is less than 1.8, additional washing (step 9) may improve 230nm/260nm ratio.

◆ **Gram positive bacteria (ex, *Bacillus subtilis*)**

Before Starting

Prepare Lysozyme Buffer (20 mM Tris-Cl (pH 8.0), 2 mM EDTA, 1.2% Triton X-100, 20 mg/ml lysozyme)

1. Pellet 1.5 ml of bacterial culture by centrifugation (8,000 rpm for 5 min).
   Wash cell pellet with 500 μl PBS or Cell Resuspension Buffer..

2. Add 200 μl of Lysozyme Buffer and then mix well by vortexing.
   [optional] for RNase A treatment : Add 1 μl of RNase A (10 mg/ml) at this step.

3. Incubate for more than 30 min at 37℃.

4. Add 400 μl of gDNA Lysis Buffer (G2) and 20 μl Proteinase K and then mix by vortexing.

5. Incubate for 15min at 70℃ and cooling.
   It is very important to lyse cells completely.
   To help lyse cells, mix by vortexing the tube every 2 min during the incubation.

6. Mix well by vortexing or pipetting.
   This step is semi-homogenization step to denature proteins. In addition, this step conduces to pass efficiently cell lysates through a column.

7. Transfer mixture on to the spin column.

8. Centrifuge for 1 min at 13,000 rpm.
   The cell lysates is very sticky at this step. If remaining solution is present in column after centrifugation, additional centrifugation is recommended to remove all traces of cell lysates.

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10. Wash the column with 750 μl of Wash Buffer and centrifugation for 1 min at 13,000 rpm.

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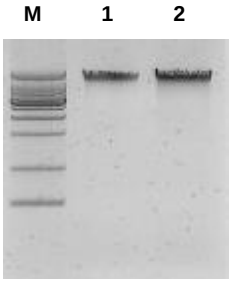
13. To elute DNA, add 50～100 μl of 55℃ pre-warmed Elution Buffer or H_2O on the center of spin column, wait for 1-5 min, and then centrifuge for 1 min at 13,000 rpm.

14. Store purified genomic DNA at -20℃ or below for longer storage.

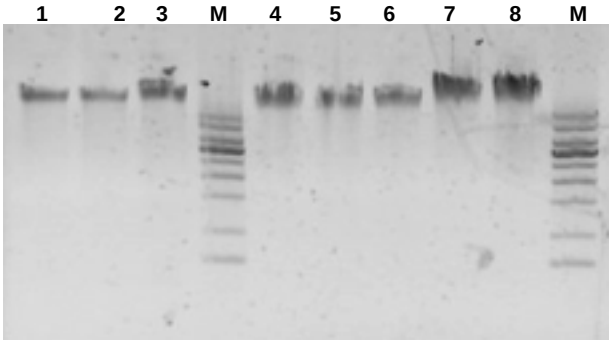
If 230nm/260nm ratio of purified DNA is less than 1.8, additional washing (step 10) may improve 230nm/260nm ratio.

Fig.1. Purification of genomic DNA from *E.coli* XL-1 Blue : 100 μl Elution process



Analyzed on a 0.7% agarose
M : 1Kbp DNA ladder marker (EBM-1002)
Lane 1 : Purified gDNA from 1 ml cultured cell (5 μl load)
2 : Purified gDNA from 1 ml cultured cell (10 μl load)

Fig 2. Culture volume vs. yield



Analyzed on a 0.7% agarose
M : 1Kbp DNA ladder marker (EBM-1002)
Lane 1. 2. 4. 5. 6. Purified gDNA from 1 ml cultured cell (5 μl load)
3. 7. 8. Purified gDNA from 1.5 ml cultured cell (5 μl load)

Customer & Technical Services

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

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