

Ver. 2404-03

F711-1(Rev.0)

# PureHelix<sup>™</sup> Genomic DNA Prep Kit [Bacteria, Animals, Plants]

(Ver.3.0), Column type

PureHelix <sup>™</sup> <i>Genomic</i> DNA Prep Kit [Bacteria, Animals, Plants]					
Cat. No.	GCTN50 (50 preps)	GCTN100 (100 preps)	GCTN200 (200 preps)		
NGD1	15ml	30ml	60ml		
NPS2	15ml	30ml	60ml		
EB	5ml	10ml	20ml		
WB	11ml (Add ethanol* 44ml)	22ml (Add ethanol* 88ml)	44ml (Add ethanol* 176ml)		
MaxBinder <sup>TM</sup> Solution	5ml	10 ml	20ml		
Nanozyme Mix	2ea (Dry)	4ea (Dry)	8ea (Dry)		
Cell Resuspension Solution	3ml	6ml	12ml		
Proteinase K (10 mg/ml)	0.5ml (Dry)	0.5ml (Dry) x 2ea	0.5ml (Dry) x 4ea		
Column Set (with cap, 50ea/Blue Box)	1box	2box	4box		
Instructions for Use	1ea	1ea	1ea		

# **Kit Contents**

\* Absolute ethanol (96-100%) does not supplied in this kit.

# Description

**PureHelix<sup>™</sup>** *Genomic DNA* **Prep Kit [Bacteria, Animals, Plants]** is designed for rapid and pure isolation of total DNA from bacteria [Gram (+) or Gram (-)], animal tissues, or plant tissues. The spin column based method completely removes PCR inhibitors such as divalent cations and proteins resulting in a high purity preparation of genomic DNA. There is no use of phenol or chloroform, handling is safe and does not produce any harmful waste. DNA purified with this kit is suitable for a variety of applications, including PCR amplification, digestion with restriction endonucleases and membrane hybridizations.



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# **Quality Control**

By Nanohelix's ISO 13485-certified quality management system, each lot of **PureHelix™** *Genomic DNA* **Prep Kit [Bacteria, Animals, Plants]** was tested against predetermined specifications to ensure consistent product quality.

# Protocol

# <Bacteria>

### Important things to do before starting

- Before using **WB**, add **absolute ethanol** according to the bottle label to obtain a working solution. You may use 80% ethanol, instead of WB. **Ethanol does not supplied in this kit.**
- Add **0.5 ml** of distilled water to the provided **Proteinase K** tube for making **10 mg/ml** concentration, and then store at -20°C.
- Add 1.5 ml of Cell Resuspension Solution to provided NanoZyme mix tube, and then Store at -20°C.

# 1. Cell Lysis

 Harvest 1 ml of cultured bacterial cells (containing 1 x 10<sup>9</sup>) by centrifuge at 12,000 rpm for 1 min. Discard the supernatants, and vigorously vortex.

 $\times$  After discarding the supernatant, the remaining 10-50  $\mu l$  of media broth doesn't affect the next purification step. The vigorous vortexing will resuspend the pelleted cells in the remaining media and help the cell lysis.

- 2) Add 50 µl of Nanozyme Mix and vortex vigorously for 30-60 sec. Incubate at 37°C for 10 min.
- Add 300 μl of NGD1 and 8 μl of Proteinase K (10 mg/ml) and mix by pipetting. Incubate at 60°C for 10 min, and then cool the tube on ice for 5 min.
- 4) Add **300 µl of NPS2**. Vortex briefly.
- 5) Place the tube on ice for 5 min, and centrifuge for 5 min at 12,000 rpm.
- 6) Transfer 600  $\mu$ l of the supernatant into a clean 1.5ml tube.
- 7) Add **200 μl of absolute ethanol** and vortex vigorously.

### 2. Column Activation [Optional]

### **\*** These steps are required for the best yield.

- 1) Place a Spin Column into a 2 ml collection tube.
- 2) Add 100  $\mu l$  of MaxBinder<sup>TM</sup> Solution into the Spin Column.
- Centrifuge at 12,000 rpm for 30 sec and immediately proceed to next step.
   You need not discard the flow-through from the collection tube.

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### 3. Loading

- 1) Pipet **400 µl of the mixture** from step 1 (Cell Lysis) into a spin column sitting in a 2 ml collection tube.
- 2) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.
- 3) Pipet the remains of the mixture from step 1 into the spin column.
- 4) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.

# 4. Washing

- 1) Add 500 µl of WB (80% ethanol) into the spin column.
- 2) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.※ Repeat these steps for high-purity DNA preparation.
- 3) Centrifuge at 12,000 rpm for 2 min to remove residual ethanol.

### 5. Elution

- Discard the 2 ml collection tube and place the spin column in a clean 1.5 ml tube.
   Add 40-50 μl of EB or distilled water into the center of the spin column.
- Centrifuge at 12,000 rpm for 2 min. Discard the spin column.
   Store the eluted DNA at 4°C or -20°C.

# <Animal tissues>

### Important things to do before starting

- It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purify. Amount of **30~50 mg animal tissue** sample can generally be processed.
- Before using **WB**, add **absolute ethanol** according to the bottle label to obtain a working solution. You may use 80% ethanol, instead of WB. Ethanol does not supplied in this kit.
- Add **0.5 ml** of distilled water to the provided **Proteinase K** tube for making **10 mg/ml** concentration, and then store at -20°C.
- Add 1.5 ml of Cell Resuspension Solution to provided NanoZyme mix tube, and then Store at -20°C.

### 1. Cell Lysis

- Add 300 µl of NGD1 and 50 µl of Nanozyme Mix to 30~50 mg of ground tissue in a 1.5 ml microcentrifuge tube. Vortex vigorously for 30-60 sec.
   We recommend grinding the tissue sample with liquid nitrogen. Immediately transfer the ground tissue into a 1.5 ml microcentrifuge tube cooled by liquid nitrogen.
- Add 8 μl of Proteinase K (10 mg/ml) and mix by pipetting.
   Incubate at 60°C for 20 min, and then cool the tube on ice for 5 min.
- 3) Add **300 µl of NPS2**. Vortex briefly.
- 4) Place the tube on ice for 5 min, and centrifuge for 5 min at 12,000 rpm.
  ※ The precipitate will be a tight pellet. If the pellet is not tight, repeat this step.

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- 5) Transfer 600 µl of the supernatant into a clean 1.5ml tube.
- 6) Add **200 µl of absolute ethanol** and vortex vigorously.

### 2. Column Activation [Optional]

#### **\*** These steps are required for the best yield.

- 1) Place a Spin Column into a 2 ml collection tube.
- 2) Add **100 µl of MaxBinder<sup>™</sup> Solution** into the Spin Column.
- Centrifuge at 12,000 rpm for 30 sec and immediately proceed to next step.
   You need not discard the flow-through from the collection tube.

### 3. Loading

- 1) Pipet **400 µl of the mixture** from step 1 (Cell Lysis) into a spin column sitting in a 2 ml collection tube.
- 2) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.
- 3) Pipet **the remains of the mixture** from step 1 into the spin column.
- 4) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.

### 4. Washing

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- 2) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.

#### **※** Repeat these steps for high-purity DNA preparation.

3) Centrifuge at 12,000 rpm for 2 min to remove residual ethanol.

### 5. Elution

- Discard the 2 ml collection tube and place the spin column in a clean 1.5 ml tube.
   Add 40-50 μl of EB or distilled water into the center of the column.
- Centrifuge at 12,000 rpm for 2 min. Discard the spin column.
   Store the eluted DNA at 4°C or -20°C.

### <Plant tissues>

### Important things to do before starting

- It is essential to use the correct amount of starting material in order to obtain optimal DNA yield and purify. Amount of **30~50 mg plant tissue** sample can generally be processed.
- Before using **WB**, add **absolute ethanol** according to the bottle label to obtain a working solution. You may use 80% ethanol, instead of WB. Ethanol does not supplied in this kit.
- Add **0.5 ml** of distilled water to the provided **Proteinase K** tube for making **10 mg/ml** concentration, and then store at -20°C.
- Add 1.5 ml of Cell Resuspension Solution to provided NanoZyme mix tube, and then Store at -20°C.

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# 1. Cell Lysis

- Add 300 µl of NGD1 and 50 µl of Nanozyme Mix to 30~50 mg of ground tissue in a 1.5 ml microcentrifuge tube. Vortex vigorously for 30-60 sec.
   We recommend grinding the tissue sample with liquid nitrogen, Immediately transfer the ground tissue into a 1.5 ml microcentrifuge tube cooled by liquid nitrogen.
- Add 8 μl of Proteinase K (10 mg/ml) and mix by pipetting. Incubate at 60°C for 20 min, and then cool the tube on ice for 5 min.
- 3) Add 300  $\mu l$  of NPS2. Vortex briefly.
- 4) Place the tube on ice for 5 min, and centrifuge at 12,000 rpm for 10 min.
- 5) Transfer 600  $\mu$ l of the supernatant into a clean 1.5ml tube.
- 6) Add **200 μl of absolute ethanol** and vortex vigorously.

# 2. Column Activation [Optional]

### **\*** These steps are required for the best yield.

- 1) Place a Spin Column into a 2 ml collection tube.
- 2) Add **100 µl of MaxBinder<sup>™</sup> Solution** into the Spin Column.
- Centrifuge at 12,000 rpm for 30 sec and immediately proceed to next step.
   You need not discard the flow-through from the collection tube.

### 3. Loading

- 1) Pipet **400 µl of the mixture** from step 1 (Cell Lysis) into a spin column sitting in a 2 ml collection tube.
- 2) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.
- 3) Pipet **the remains of the mixture** from step 1 into the spin column.
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### 4. Washing

- 1) Add 500 µl of WB (80% ethanol) into the spin column.
- 2) Centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.

### **※** Repeat these steps for high-purity DNA preparation.

3) Centrifuge at 12,000 rpm for 2 min to remove residual ethanol.

### 5. Elution

- Discard the 2 ml collection tube and carefully place the spin column in a clean 1.5 ml tube.
   Add 40-50 μl of EB or distilled water into the center of the column.
- Centrifuge at 12,000 rpm for 2 min. Discard the spin column.
   Store the eluted DNA at 4°C or -20°C.

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# Products

Cat. No.	Products	Size
GCTN50	PureHelix™ <i>Genomic</i> DNA Prep Kit [Bacteria, Animals, Plants] (Ver. 3.0) Column type	50 preps
GCTN100	PureHelix™ <i>Genomic</i> DNA Prep Kit [Bacteria, Animals, Plants] (Ver. 3.0) Column type	100 preps
GCTN200	PureHelix <sup>™</sup> <i>Genomic</i> DNA Prep Kit [Bacteria, Animals, Plants] (Ver. 3.0) Column type	200 preps

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