

PureHelix™ Genomic DNA Prep Kit [Bacteria]

Solution Type

Kit Contents

PureHelix™ Genomic DNA Prep Kit [Bacteria]		
Cat. No.	GSBA100 (100preps)	GSBA400 (400preps)
Cell Lysis Solution	30ml	120ml
Cell Resuspension Solution	30ml	60ml x 2ea
DNA Hydration Solution	10ml	40ml
Protein Precipitation Solution	10ml	40ml
WB	11ml (Add 44ml ethanol)	44ml (Add 176ml ethanol)
RNase A (4mg/ml)	0.2ml (Dry)	0.8ml (Dry)
Lysozyme (100mg/ml)	0.25ml (Dry)	1ml (Dry)
Instructions for Use	1ea	1ea

Description

PureHelix™ Genomic Prep Kit [Bacteria] is designed for high-yield and high-quality isolation of genomic DNA from gram-negative and gram-positive bacteria samples. This solution based system minimizes DNA fragmentation that may be problematic in spin-column/filtration based methods. Because phenol or chloroform is not used it 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.

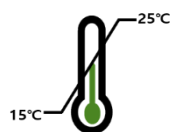
Applications

Genome sequencing

Southern blot analysis

PCR, quantitative real-time PCR

Storage



Room temperature

Shelf life



12 months

Quality Control

Each lot of **PureHelix™ Genomic Prep Kit [Bacteria]** was tested against predetermined specifications to ensure consistent product quality.

Protocol

<Gram-negative bacteria>

Important things to do before starting

- Prepare **Isopropanol** (2-propanol) (**not included in this kit**).
- 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.
- **For 100 Prep Kit (GSBA100),**
Add **0.2 ml** of distilled water into the **RNase A tube** to make 4mg/ml concentration, and then stored at -20°C.
- **For 400 Prep Kit (GSBA400),**
Add **0.8 ml** of distilled water into the **RNase A tube** to make 4mg/ml concentration, and then stored at -20°C.

Cell Lysis

1. Transfer 1 ml of cultured cells into a 1.5 ml microcentrifuge tube.
2. Harvest the cells centrifuge at 12,000 rpm for 1 min, and discard the supernatant.
3. Resuspend the cell pellet in **300 µl** of **Cell Lysis Solution**.

RNase Treatment

4. Add **1.5 µl** of **RNase A (4mg/ml)** and mix by inverting.
5. Incubate at 37°C for 15 min and cool on ice for 1 min.

Protein Precipitation

6. Add **100 µl** of **Protein Precipitation Solution**, and vortex briefly.
7. Centrifuge at 12,000 rpm for 5 min.

DNA Precipitation

8. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube containing **300 µl** of **Isopropanol** (2-propanol), and mix the sample by inverting gently 50 times.
9. Centrifuge at 12,000 rpm for 1 min. Discard the supernatant and drain the tube briefly on clean absorbent paper.
※ **DNA should be visible as a small white pellet.**
10. Add **500 µl** of **WB (80% ethanol)** and invert the tube several times to wash the DNA pellet.

11. Centrifuge at 12,000 rpm for 1 min. Discard the ethanol carefully and air dry at room temperature for 10 min.

※ **The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet. Ethanol should be completely removed, but DNA is very difficult to redissolve when over-dried.**

DNA Hydration

12. Add **20-100 µl** of **DNA Hydration Solution** to the dried DNA pellet. Hydrate the DNA by incubating at 65°C for 30 min. Store the DNA at 4°C.

※ **For long time storage, Place the sample at -20°C or -80°C.**

<Gram-positive bacteria>

Important things to do before starting

- Prepare **Isopropanol** (2-propanol) (**not included in this kit**).
- 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.
- **For 100 Prep Kit (GSBA100)**
Add **0.2 ml** of distilled water into the **RNase A tube** to make 4mg/ml concentration, and then stored at -20°C.
Add **0.25 ml** of distilled water into the **Lysozyme tube** to make 100mg/ml concentration, and then stored at -20°C.
- **For 400 Prep Kit (GSBA400)**
Add **0.8 ml** of distilled water into the **RNase A tube** to make 4mg/ml concentration, and then stored at -20°C.
Add **1.0 ml** of distilled water into the **Lysozyme tube** to make 100mg/ml concentration, and then stored at -20°C.

Cell Lysis

1. Transfer 1 ml of cultured cells into a 1.5 ml microcentrifuge tube.
2. Harvest the cells by centrifuge at 12,000 rpm for 1 min, and discard the supernatant. Resuspend the cell pellet in **300 µl** of **Cell Resuspension Solution**.
3. Add **2 µl** of **Lysozyme (100mg/ml)** and mix well by inverting. Incubate the tube at 37°C for 1 hour. Centrifuge at 12,000 rpm for 1 min, and discard the supernatant. Resuspend the cell pellet in **300 µl** of **Cell Lysis Solution**.
4. Add **1.5 µl** of **RNase A (4mg/ml)** and mix by inverting. Incubate at 37°C for 15-60 min and cool on ice for 1 min.

Protein Precipitation

5. Add **100 µl** of **Protein Precipitation Solution**, and vortex briefly.
6. Centrifuge at 12,000 rpm for 5 min.

DNA Precipitation

7. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube containing **300 µl** of **Isopropanol** (2-propanol). Mix the sample by inverting gently 50 times.
8. Centrifuge at 12,000 rpm for 1 min. Discard the supernatant and drain the tube briefly on clean absorbent paper.
※ **DNA should be visible as a small white pellet.**
9. Add **500 µl** of **WB (80% ethanol)** and invert the tube several times to wash the DNA pellet.
10. Centrifuge at 12,000 rpm for 1 min. Discard the ethanol carefully and air dry at room temperature for 10 min.
※ **The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet. Ethanol should be completely removed, but DNA is very difficult to redissolve when over-dried.**

DNA Hydration

11. Add **20-100 µl** of **DNA Hydration Solution** to the dried DNA pellet. Hydrate the DNA by incubating at 65°C for 30 min. Store the DNA at 4°C.
※ **For long time storage, Place the sample at -20°C or -80°C.**

Products

Cat. No.	Products	Size
GSBA100	PureHelix™ <i>Genomic</i> DNA Prep Kit [Bacteria] (Solution Type)	100preps
GSBA400	PureHelix™ <i>Genomic</i> DNA Prep Kit [Bacteria] (Solution Type)	400preps