Yeastern Biotech Co., Ltd

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Ver. R0426

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Genomic DNA Extraction Kit (Bacteria/Fungi)

Cat. No.
SYG115-001
FYG115-100

Genomic DNA Extraction Kit (Bacteria/Fungi)

(SYG115-001/ FYG115-100) Store at RT/4 ℃ Ver. R0426

Sample：10^9 Bacteria
5 x 10^7 Fungus cells

Yield： Up to 50 µg

Contents

Item	SYG115-001 (4 preps)	FYG115-100 (100 preps)
N1 Buffer	4 ml	100 ml
N2 Buffer	1.5 ml	35 ml
N3 Buffer	2 ml	45 ml
W1 Buffer	2 ml	45 ml
W2 Buffer	300 ul x2	15 ml
Elution Buffer	1 ml	10 ml
GZ Column	4 pcs	100 pcs
Collection Tube	4 pcs	100 pcs
User Manual	1	1

Buffer Preparation

- **Add ethanol (96-100%) to the W2 Buffer prior to first use.**

Buffer	SYG115-001 (4 preps)	FYG115-100 (100 prep)
W2 Buffer Ethanol (96 - 100%)	300 ul x2 1.2 ml x2	15 ml 60 ml

Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add ethanol (96- 100 %) to W2 Buffer when first open.
3. Prepare dry bath or water bath before the operation.
4. Resolve any precipitate by warming at 37°C.

Additional Requirements

For Gram-positive bacteria sample:

a. Lysozyme Buffer
   - (20 mg/ml lysozyme; 20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100, pH 8.0) for Gram-Positive Bacteria Sample. Prepare the Lysozyme Buffer fresh immediately prior to use.

For Fungus sample:

a. Lyticase or Zymolase
b. Sorbitol Buffer (1.2 M sorbitol; 10 mM CaCl2; 0.1M Tris-Cl pH 7.5; 35 mM mercaptoethanol)

Description

The Genomic DNA Extraction Kit (Bacteria/Fungi) is designed for rapid extraction of pure genomic DNA from bacteria and fungus cells. Efficiently remove cellular debris and inhibitors, this kit using column-type tube in purification process through three simple steps of binding, washing and then elution for the safe and convenient extraction of high-purity genomic DNA. The entire process can be completed in less than 1 hour without phenol/chloroform, and the final product can be used in PCR or other downstream experiments.

Purification Protocols

Step 1. Sample preparation

For Gram-Positive Bacteria

a. Transfer up to 10^9 cultured gram-positive bacterial cells to a microcentrifuge tube (not provided).
b. Centrifuge 1 minute at 14,000 x g. Remove the supernatant completely.
c. Resuspend the cells with 100 µl Lysozyme Buffer by pipetting.
d. Incubate at room temperature for 20 minutes.

For Gram-Negative Bacteria

a. Transfer up to 10^9 cultured gram-negative bacterial cells to a microcentrifuge tube (not provided).
b. Centrifuge 1 minute at 14,000 x g. Remove the supernatant completely.
c. Resuspend the cells with 50 µl N1 Buffer by pipetting.

For Fungus Cells

a. Transfer up to 5 x 10^7 fungus cells to a microcentrifuge tube (not provided).
b. Centrifuge 5 minutes at 6,000 x g. Remove the supernatant completely.
c. Resuspend the cells with 600 µl Sorbitol Buffer by pipetting.
d. Add 200 U of Lyticase or Zymolase.
e. Incubate at 30°C for 30 minutes.
f. Centrifuge the mixture for 10 minutes at 2,000 x g, and remove the supernatant completely.
g. Resuspend the cells in 50 µl N1 Buffer by pipetting.

Step 2. Cell Lysis

a. Add 300 µl of N2 Buffer to the sample and mix thoroughly by vortexing.
b. Incubate the sample at 60 °C for 10 minutes until the sample lysate is clear. Invert the tube every 3 minutes during incubation.
c. Pre-heat the Elution Buffer at 75 °C.

Optional step:

a. Add 5 µl of RNase A (10mg/ml, not provided) to sample lysate and mix by vortexing.
b. Incubate at room temperature for 5 minutes.

If RNA-free genomic DNA is required, perform this optional step.

Step 3. Protein Removal

a. Add 400 µl N3 Buffer to the sample and shake vigorously.
b. Centrifuge 14,000 x g for 1 minute.

Step 4. DNA Binding

a. Place the GZ Column with a Collection Tube.
b. Transfer the sample mixture to the GZ Column and centrifuge at 14,000 x g for 30 seconds.
c. Discard the flow-through and place the GZ Column back in the Collection Tube.

Step 5. Wash

a. Add 400 µl of W1 Buffer to the GZ Column.
b. Centrifuge at 14,000 x g for 30 seconds.
c. Discard the flow-through and place the GZ Column back in the Collection Tube.
d. Add 600 µl of W2 Buffer (ethanol added) to the GZ Column.
e. Centrifuge at 14,000 x g for 30 seconds.
f. Discard the flow-through and return the GZ Column back to the Collection Tube.
Note: Make sure that ethanol (96-100%) has been added into W2 Buffer when first use.

Step 6. Dry column

a. Centrifuge at 14,000 x g for 3 minutes to dry the GZ column.

Step 7. Elution

a. Combine the GZ Column with a 1.5 ml eppendorf (not provided).
b. Add 50-90 µl of preheated Elution Buffer (75 °C) into the center of the column matrix.
c. Stand 75 °C for 3 minutes.
d. Centrifuge at 14,000 x g for 2 minutes to elute purified DNA.

Step 7. Store DNA

a. Store the DNA fragment at 4°C or -20°C.

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