Yeastern Biotech Co., Ltd

Genomic DNA Extraction Mini Kit (Blood and Urine)

Product Use Limitation & Warranty

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Ver. Q0306

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Cat. No.
SYG109-002
FYG109-100

Genomic DNA Extraction Mini Kit (Blood and Urine)

(SYG109-002/FYG109-100)　　Store at RT/4 ℃　Ver. Q0306

Sample：300 µl（Whole Blood）/200 µl Buffy Coat /Urine　　**Yield**：Up to 50 µg

Contents

	SYG109-002	FYG109-100
B1 Buffer	4 ml	100 ml
B2 Buffer	1.5 ml	35 ml
B3 Buffer	0.5 ml	12 ml
BC Buffer	2 ml	45 ml
W1 Buffer	2 ml	45 ml
W2 Buffer*	0.3 ml x2	15 ml
Elution Buffer	1 ml	10 ml
BZ Column	4 pcs	100 pcs
Collection Tube	4 pcs	100 pcs
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Buffer Preparation

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

	SYG109-002	FYG109-100
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.

Purification Protocols

I. For Blood：

Step 1. Sample preparation

a. Transfer up to 300 µl of whole Blood or 200 µl Buffy Coat to a microcentrifuge tube (not provided).(If sample volume is less than 200 µl, adjust the volume to 200 µl with PBS).

Step 2. RBC Lysis

a. Add **900 µl B1 Buffer** to the tube and mix by inversing the tube. Incubate the mixture at room temperature for 10 minutes. During incubation, invert the tube every 5 minutes.

Step 3. Cell Lysis

a. Centrifuge 5 mins at 4000 x g. Remove the supernatant completely.
b. Resuspend the cell with **50 µl B1 Buffer** by pipetting the pellet.
c. **Add 300 µl B2 Buffer** to the sample and mix thoroughly by vortexing.
d. Incubate at 60 °C for 10 mins until the sample lysate is clear. Invert the tube every 3 minutes during incubation.
e. Preheat required Elution Buffer (50-90 µl per sample) in a 60 °C water bath.
f. Centrifuge 5 mins at 3000 x g. Remove the supernatant completely.

Optional Step

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

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

Step 4. Protein Removal

a. **Add 100 µl B3 Buffer** to the sample and vortex immediately for 10 seconds.
b. Incubate on ice for 5 mins.
c. Centrifuge at 14,000 x g for 3 minutes.
d. Transfer the supernatant to a clear 1.5 ml microcentrifuge tube.

Step 5. DNA Binding

a. Add **400 µl BC Buffer to sample and shake vigorously.**
b. Place a B**Z Column** to a Collection Tube. Transfer the sample mixture to BZ Column.
c. Centrifuge at full speed (13,000 rpm/17,900x g) for 30 seconds.
d. Discard the flow-through and place BZ Column back in the Collection Tube.

Step 6. Wash

a. **Add 400 µl W1 Buffer** to BZ Column.
b. Centrifuge at full speed (13,000 rpm/17,900x g) for 30 seconds. Discard the flow-through and place BZ Column back in the Collection Tube.
c. **Add 600 µl W2 Buffer** (ethanol added) to BZ Column.
d. Centrifuge at full speed (13,000 rpm/17,900x g) for 30 seconds. Discard the flow-through and place BZ Column back in the Collection Tube.

Step 7. Dry

a. Centrifuge at full speed (13,000 rpm/17,900x g) for 2 minutes to dry the column.

Step 8. Elution

a. Place BZ Column to a clean 1.5 ml microcentrifuge tube (not provided).
b. **Add 50-90 µl of pre-heated Elution Buffer (60** °C**)** into the center of the column matrix.
c. Stand at **60 °C** for 5 minutes.
d. Centrifuge at full speed (13,000 rpm/17,900x g) for 2 minutes to elute purified DNA. Store the DNA fragment at 4 °C or -20 °C.

II. For Urine：

Step 1. Sample preparation

a. Centrifuge the urine sample at 4000 x g for 5 minutes.
b. Remove the supernatant completely and resuspend the pellet in **900 µl B1 Buffer** by pipetting.

Step 2. Cell Lysis

a. Centrifuge 5 mins at 4000 x g. Remove the supernatant completely.
b. Resuspend the cell with **50 µl B1 Buffer** by pipetting the pellet.
c. **Add 300 µl B2 Buffer** to the sample and mix thoroughly by vortexing.
d. Incubate at 60 °C for 10 mins until the sample lysate is clear. Invert the tube every 3 minutes during incubation.
e. Preheat required Elution Buffer (50-90 µl per sample) in a 60 °C water bath.
f. Centrifuge 5 mins at 3000 x g. Remove the supernatant completely.

Optional Step

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

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

Step 3. Protein Removal

a. **Add 100 µl B3 Buffer** to the sample and vortex immediately for 10 seconds.
b. Incubate on ice for 5 mins.
c. Centrifuge at 14,000 x g for 3 minutes.
d. Transfer the supernatant to a clear 1.5 ml microcentrifuge tube.

Step 4. DNA Binding

a. Add **400 µl BC Buffer to sample and shake vigorously.**
b. Place a **BZ Column** to a Collection Tube. Transfer the sample mixture to BZ Column.
c. Centrifuge at full speed (13,000 rpm/17,900x g) for 30 seconds.
d. Discard the flow-through and place BZ Column back in the Collection Tube.

Step 5. Wash

a. **Add 400 µl W1 Buffer** to BZ Column.
b. Centrifuge at full speed (13,000 rpm/17,900x g) for 30 seconds. Discard the flow-through and place BZ Column back in the Collection Tube.
c. **Add 600 µl W2 Buffer** (ethanol added) to BZ Column.
d. Centrifuge at full speed (13,000 rpm/17,900x g) for 30 seconds. Discard the flow-through and place BZ Column back in the Collection Tube.

Step 6. Dry

a. Centrifuge at full speed (13,000 rpm/17,900x g) for 2 minutes to dry the column.

Step 7. Elution

a. Place BZ Column to a clean 1.5 ml microcentrifuge tube (not provided).
b. **Add 50-90 µl of pre-heated Elution Buffer (60 °C)** into the center of the column matrix.
c. Stand at **60 °C** for 5 minutes.
d. Centrifuge at full speed (13,000 rpm/17,900x g) for 2 minutes to elute purified DNA. Store the DNA fragment at 4 °C or -20 °C.

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