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Ver. R0426

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Total RNA Kit (Blood/ Bacteria)

Cat. No.
SYG306-001
FYG306-100
FYG306-300

Total RNA kit (Blood/ Bacteria)

(SYG306-001/FYG306-100/FYG306-300)

Store at RT/4 ℃ Ver. R0426

Sample：10^9 x Bacteria Cells / 300 µl of blood **Yield**：Up to 30 µg

Contents

	SYG306-001	FYG306-100	FYG306-300
BR1 Buffer	4 ml	110 ml	100 ml x 3
BR2 Buffer	2 ml	45 ml	125 ml
BR3 Buffer	1 ml	25 ml	65 ml
W1 Buffer	2 ml	45 ml	125 ml
W2 Buffer	300 µl x2	15 ml	25 ml x2
Elution Buffer	1 ml	10 ml	30 ml
RZ Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
User Manual	1	1	1

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

	SYG306-001	FYG306-100	FYG306-300
W2 Buffer	300 ul x2	15 ml	25 ml x2
Ethanol (96 - 100%)	1.2 ml x2	60 ml	100 ml x2

Additional Requirements

1. ß – Mercaptoethanol
2. Lysozyme Buffer (20 mg/ ml lysozyme)
3. RNase-free microcentrifuge tubes
4. 70% Ethanol

Important Notes

1. Buffer contains chaotropic salt is harmful and irritant agent.
2. Use sterile, RNase-free pipet tips and microcentrifuge tubes. Wear a lab coat and disposable gloves to prevent RNase contamination.
3. Make sure the starting sample amount is under the limit.
4. Add ethanol (96-100%) to W2 Buffer prior to the initial use.
5. All purification steps should be carried out at room temperature.
6. All centrifugations should be carried out in a table-top microcentrifuge at >12000 × g (10,000-14,000 rpm, depending on the rotor type).

Step 1. Sample preparation

I. For Gram- Bacteria：

a. Transfer up to 10^9 bacteria cells to a microcentrifuge tube.
b. Centrifuge at 12,000 xg for 1 min and remove the supernatant completely.
c. **Add 200 µl of BR3 Buffer** to the tube and resuspend the cell pellet by vortexing or pipetting.
d. Incubate at room temperature for 5 minutes.
e. **Add 300 µl of BR2 Buffer** and 3 µl ß-Mercaptoethanol, mix by vortexing.
f. Incubate at room temperature for 5 minutes.

II. For Gram+ Bacteria：

a. Transfer up to 10^9 bacteria cells to a microcentrifuge tube.
b. Centrifuge at 12,000 xg for 1 min and remove the supernatant completly.
c. **Add 200 µl of Lysozyme Buffer** (20 mg/ml lysozyme) to the tube and resuspend the cell pellet by vortexing or pipetting.
d. Incubate at room temperature for 10 minutes.
e. **Add 300 µl of BR2 Buffer** and 3 µl ß-Mercaptoethanol, mix by vortexing.
f. Incubate at room temperature for 5 minutes.

III. For Blood：

a. Transfer up to 300 µl of blood to a RNase-free microcentrifuge tube.
b. **Add 900 µl of BR1 Buffer**, then mix by inverting.
c. Incubate the mixture on ice for 10 minutes, and invert every 5 minutes.
d. Centrifuge at 4℃ at 4,000 xg for 5 minutes.
e. Remove the supernatant completely.
f. **Add 100 µl of BR1 Buffer** and resuspend the pellet by pipetting.
g. **Add 400 µl of BR2 Buffer** and 4 µl ß-Mercaptoethanol, mix by vortexing.
h. Incubate at room temperature for 5 minutes.

Step 2. Cell Lysis

a. Centrifuge at 14,000 x g for 10 minutes.
b. Transfer the supernatant to a clean microcentrifuge tube.

Step 3. RNA Binding

a. **Add 500 µl of 70% ethanol** and shake vigorously.
b. Place a **RZ Column** in **Collection Tube**.
c. Transfer the sample mixture (up to 700 µl once) to **RZ Column** and centrifuge 1 minute at 14,000 xg.
d. Discard the flow-through and place **RZ Column** back in the **Collection Tube**.

Step 4-1. Washing

a. **Add 400 µl W1 Buffer** to **RZ Column**.
b. Centrifuge at 14,000 x g for 30 seconds.
c. Discard the flow-through and place **RZ Column** back in the **Collection Tube**.

Step 4-2. Washing

a. **Add 600 µl of W2 Buffer (ethanol added)** into the column.
b. Centrifuge at 14,000 x g for 30 seconds.
c. Discard the flow-through and place the **RZ Column** back in the **Collection Tube**.

Step 5. Drying

a. Centrifuge at 14,000 rpm for 2 minutes to dry the column matrix.

Step 6. RNA Elution

a. Place **RZ Column** to a clean 1.5 ml microcentrifuge tube.
b. **Add 50-90 µl of preheated Elution Buffer** (75°C) into the center of the column matrix.

Step 7. Purify RNA

a. Stand at room temperature for 3 minutes.
b. Centrifuge at 14,000 xg for 2 minutes to elute purified RNA.

Step 8. Store RNA

a. Store the RNA fragment at -80°C.

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