



Product Use Limitation & Warranty

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Ver. Q0502

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Address: 6F-3, 23 Lane 169, Kang Ning St., Shijr, Taipei, 22180 Taiwan.
Tel: +886-2-2695-3922 **Fax:** +886-2-2695-3979
Email: yeastern@yeastern.com **Website:** www.yeastern.com

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Total RNA Kit (Tissue)2.0

Cat. No.
SYG307-001
FYG307-100
FYG307-300

Total RNA Kit (Tissue)2.0

(SYG307-001/ FYG307-100/ FYG307-300) Store at RT/4 °C Ver. Q0502

Sample : 30 mg of fresh animal tissue

Yield : Up to 30 µg

25 mg of paraffin-embedded tissue

Contents

| Items | SYG307-001 (4 preps) | FYG307-100 (100 preps) | FYG307-300 (300 preps) |
|-------------------------|-------------------------|---------------------------|---------------------------|
| TR Buffer | 2 ml | 45 ml | 125 ml |
| W1 Buffer | 2 ml | 45 ml | 125 ml |
| W2 Buffer | 300 ul x2 | 15 ml | 25 ml x2 |
| Elution Buffer | 1 ml | 10 ml | 30 ml |
| DNase Incubation Buffer | 1 ml | 10 ml | 30 ml |
| Micropestle | 4 pcs (provided) | 100 pcs | 300 pcs |
| RZ Column | 4 pcs | 100 pcs | 300 pcs |
| Collection Tube | 4 pcs | 100 pcs | 300 pcs |
| User Manual | 1 | 1 | 1 |

- For FYG307-100/300, it needs to purchase the micropestle additionally.

Buffer Preparation

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

| Buffer | SYG307-001 (4 preps) | FYG307-100 (100 preps) | FYG307-300 (300 preps) |
|---------------------|-------------------------|---------------------------|---------------------------|
| W2 Buffer | 300 ul x2 | 15 ml | 25 ml x 2 |
| Ethanol (96 - 100%) | 1.2 ml x2 | 60 ml | 100 ml x 2 |

Additional Requirements

1. β - Mercaptoethanol
2. Xylene
3. RNase-free microcentrifuge tubes
4. 70% ethanol
5. Absolute ethanol
6. DNase I

Important Notes

1. Buffer contains chaotropic salt is harmful and irritant agent. Wear gloves and lab coat when handling these buffers.
2. Use sterile, RNase-free pipet tips and microcentrifuge tube. 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 when prior to the initial Use.
5. All purification steps should be carried out at room temperature.
6. All centrifugation should be carried out in a table-top microcentrifuge at $>12000 \times g$ (10,000-14,000 rpm, depending on the rotor type).

Purification Protocols

Step 1. Sample preparation

For Fresh Tissue

- a. Cut off 30 mg of fresh animal tissue.
Method A: Grind the sample under liquid nitrogen to a fine powder with pestle and mortar.
Method B: Grind the sample using micropestle (not provided, FYG905-100/300) in a microcentrifuge tube.

For Paraffin-Embedded Tissue

- a. Slice up to 25 mg of paraffin-embedded tissue and transfer to a 1.5 ml tube.
- b. Add 1 ml xylene and vortex vigorously and incubate room temperature for 10 minutes.
- c. Vortex every 2 minutes during incubation.
- d. Centrifuge 3 minutes at $14,000 \times g$. Remove the supernatant.
- e. Add 1 ml absolute ethanol (not provided) and mix by inverting.
- f. Centrifuge 3 minutes at $14,000 \times g$. Remove the supernatant.
- g. Repeat step 1 (f-g) one more time.
- h. Open the tube and incubate at the 37°C for 15 minutes.

Step 2. Cell Lysis

- a. Add 400 μ l of TR Buffer and 4 μ l β -mercaptoethanol (not provided) to the sample.
- b. Grind the sample until the sample lysate is clear.
- c. Transfer the sample mixture to a RNase-free microcentrifuge tube and incubate at 75°C for 20 mins.
- d. Pre-heat the Elution Buffer at 75 °C

Step 3. DNA Binding

- a. Add 400 μ l 70% ethanol and shake vigorously.
- b. Place RZ Column with a Collection Tube.
- c. Transfer the sample mixture (up to 700 μ l once) to the RZ Column and centrifuge at 14,000 x g for 1 minute.
- d. Discard the flow-through and place the RZ Column back in the Collection Tube.

Optional Step: DNA residue degradation

- a. Add 100 μ l DNase I (180 Units, 10 μ l DNase I + 90 μ l DNase Incubation Buffer) to the center of the RZ Column matrix.
- b. Stand for 10 minutes at room temperature.

Step 4. Wash

- a. Add 400 μ l of W1 Buffer to the RZ 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.
- d. Add 600 μ l of W2 Buffer (ethanol added) to the RZ Column.
- e. Centrifuge at 14,000 x g for 30 seconds.
- f. Discard the flow-through and return the RZ Column back to the Collection Tube.

Note: Make sure that ethanol (96-100%) has been added into W2 Buffer when first use.

Step 5. Dry column

- a. Centrifuge at 14,000 x g for 2 minutes to dry the RZ column.

Step 6. Elution

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

Step 7. Store RNA

- a. Store the RNA fragment at -80°C.

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