

# **Total RNA Kit (Plant)2.0**

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Cat. No. SYG308-001 **FYG308-100** FYG308-300

# Total RNA Kit (Plant)2.0

(SYG308-001/ FYG308-100/ FYG308-300) Store at RT/4 °C Ver. Q0125

 $\textbf{Sample}: \ 100 \ \text{mg of Tissue} \qquad \qquad \textbf{Yield}: \ \ \text{Up to 30 } \mu \text{g}$ 

25 mg of dry plant Tissue

#### Contents

Items	SYG308-001 (4 preps)	FYG308-100 (100 preps)	FYG308-300 (300 preps)
PR Buffer	4 ml	110 ml	105 ml x3
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
RZ Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
User Manual	1	1	1

# **Buffer Preparation**

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

Buffer	SYG308-001 (4 preps)	FYG308-100 (100 preps)	FYG308-300 (300 preps)
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. RNase-free microcentrifuge tubes
- 3. Isopropanol
- 4. ethanol (96-100%)

# **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 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 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

- a. Cut off 100 mg of fresh plant tissue or 25 mg of dry plant tissue.
- b. Grind the sample under liquid nitrogen to a fine powder by using a mortar and pestle.

### Step 2. Cell Lysis

- a. Add 1 ml PR Buffer and 10  $\mu$ l of  $\beta$  Mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved.
- b. Transfer the sample mixture to a RNase-free microcentrifuge tube and incubate at 75°C for 30 minutes. (Invert the tube every 10 minutes.)
- c. Centrifuge at 2-8°C at  $14,000 \times g$  for 10 minutes and transfer the supernatant to a new microcentrifuge tube.
- d. Add 1/2 volume of isopropanol to the sample and shake vigorously.
- e. Place a RZ column in Collection Tube.

#### Step 3. RNA Binding

- a. Transfer the sample mixture (up to 700  $\mu$ l once) to RZ column and centrifuge 30 seconds at 14,000 x g.
- b. Discard the flow-through and place RZ Column back in the Collection Tube.

#### Step 4. Wash

- 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.
- d. Add 600 µl W2 Buffer (ethanol added) to RZ Column.
- e. Centrifuge at 14,000 x g for 30 seconds.
- f. Discard the flow-through and place RZ Column back in the Collection Tube.

#### Step 5. Dry column

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

#### Step 6. Elution

- a. Place RZ Column to a clean 1.5 ml microcentrifuge tube (not provided).
- b. Add 50-90  $\mu$ l of Elution Buffer into the center of the column matrix.
- c. Stand at room temperature for 2 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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