Yeastern Biotech Co., Ltd

Product Use Limitation & Warranty

This product is intended to be used for life science research only. It has not been approved for drug or diagnostic purpose. YEASTERN's products should not be resold, modified for resale, or used to manufacture commercial products without written approval by YEASTERN. YEASTERN guarantees the performance of all products in the manner described in our protocol. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, YEASTERN will replace it free of charge.

Ver. Q0502

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

Copyright® 2017 All rights reserved. Yeastern Biotech Co., Ltd.

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 ℃ 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. ß - 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 × 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 x g. Remove the supernatant.
e. Add 1 ml absolute ethanol (not provided) and mix by inverting.
f. Centrifuge 3 minutes at 14,000 x 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 µl of TR Buffer and 4 µl 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 µl 70% ethanol and shake vigorously.
b. Place RZ Column with a Collection Tube.
c. Transfer the sample mixture (up to 700 µl 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 µl DNase I (180 Units, 10 µl DNase I + 90 µl 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 µl 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 µl 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 µl 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.

Product Use Limitation & Warranty

This product is intended to be used for life science research only.
It has not been approved for drug or diagnostic purpose.
YEASTERN’ s products should not be resold, modified for resale, or used to manufacture commercial products without written approval by YEASTERN. YEASTERN guarantees the performance of all products in the manner described in our protocol. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, YEASTERN will replace it free of charge.