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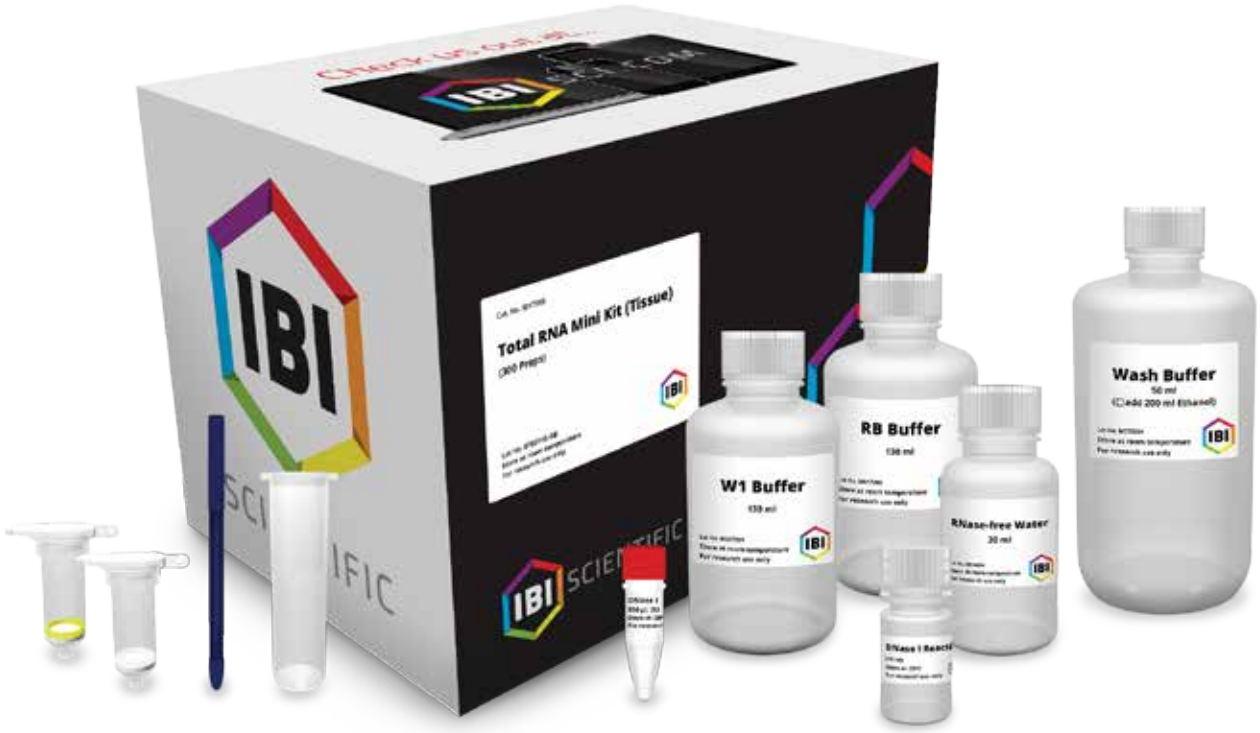


IBI SCIENTIFIC

# INSTRUCTION MANUAL

## Total RNA Mini Kit (Tissue)

IB47300 (4 prep sample kit)
IB47301 (50 prep kit)
IB47302 (100 prep kit)
IB47303 (300 prep kit)

# Advantages

**Sample:** up to 25 mg of tissue

**Yield:** 5-30 µg

**Format:** spin column

**Operation Time:** within 15 minutes

**Elution Volume:** 25-100 µl

**Kit Storage:** dry at room temperature (15-25°C)

# Introduction

The Total RNA Mini Kit (Tissue) was designed specifically for purifying total RNA from a variety of animal tissue. Samples can be efficiently homogenized in a microcentrifuge tube using the provided micropestle. Detergents and chaotropic salt are used to lyse cells and inactivate RNase with an optional in-column DNase treatment. RNA in the chaotropic salt is bound by the glass fiber matrix of the spin column. Once any contaminants have been removed, using the Wash Buffer (containing ethanol), the purified total RNA is eluted by RNase-free Water. The procedure does not require phenol extraction or alcohol precipitation and can be completed within 15 minutes. The purified RNA is ready for use in RT-PCR, Northern Blotting, Primer Extension and cDNA Library Construction.

# Quality Control

The quality of the Total RNA Mini Kit (Tissue) is tested on a lot-to-lot basis according to IBI's ISO-certified quality management system. Total RNA is isolated from a 25 mg animal tissue sample, quantified with a spectrophotometer and analyzed by electrophoresis.

# Kit Contents

| Component | IB47300 | IB47301 | IB47302 | IB47303 |
|---|---|---|---|---|
| RB Buffer | 2 ml | 30 ml | 60 ml | 130 ml |
| DNase I[1] (2U/µl) | 20 µl | 275 µl | 550 µl | 550 µl x 3 |
| DNase I Reaction Buffer | 200 µl | 2.5 ml | 5 ml | 15 ml |
| W1 Buffer | 2 ml | 30 ml | 50 ml | 130 ml |
| Wash Buffer[2] (Add Ethanol) | 1.5 ml (6 ml) | 25 ml (100 ml) | 25 ml + 12.5 ml (100 ml)(50 ml) | 50 ml x 2 (200 ml x 2) |
| RNase-free Water | 1 ml | 6 ml | 15 ml | 30 ml |
| Filter Columns | 4 | 50 | 100 | 300 |
| RB Columns | 4 | 50 | 100 | 300 |
| 2 ml Collection Tubes | 8 | 100 | 200 | 600 |
| Micropestles | 4 | 50 | 100 | 300 |

[1] DNase I is shipped at room temperature and should be stored at -20°C for extended periods after receiving the kit.

[2] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

# Order Information

| Product | Package Size | Catalogue # |
|---|---|---|
| Total RNA Mini Kit (Blood/Cell) | 50/100/300 preps | IB47321/322/323 |
| Total RNA Mini Kit (Tissue) | 50/100/300 preps | IB47301/302/303 |
| Total RNA Mini Kit (Plant) | 50/100/300 preps | IB47341/342/343 |
| rBAC Mini RNA Bacteria Kit | 100/300 preps | IB47421/422 |
| rYeast Total RNA Mini Kit | 100/300 preps | IB47411/412 |
| miRNA Isolation Kit | 100 preps | IB47371 |
| IBI Isolate | 100/200 rxns | IB47601/302 |
| IBI Tri-Isolate | 100/200 rxns | IB47631/632 |

# Caution

RB Buffer contains chaotropic salt. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

# Steps to prevent RNase contamination

Disposable and nondisposable plasticware and automatic pipettes should be sterile and used only for RNA procedures.

### IMPORTANT BEFORE USE!

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: 20-G needle syringe, absolute ethanol, ddH2O (RNase/DNase-free) to prepare 70% ethanol, microcentrifuge tubes, pipette tips, ß-mercaptoethanol

# Total RNA Mini Kit (Tissue) Protocol

<table>
<tr><td>Sample Prep.</td><td>Fresh or Frozen Animal Tissue<br>• Cut off up to 25 mg of fresh/frozen tissue and transfer to a 1.5 ml microcentrifuge tube then proceed with Cell Lysis.<br>NOTE: If using frozen animal tissue, the sample must have been flash frozen in liquid nitrogen and immediately stored at -70°C until use to avoid RNA degradation.</td></tr>
<tr><td>Step 1<br>Cell Lysis</td><td>• Add 400 µl of RB Buffer and 4 µl of ß-mercaptoethanol (or 8 µl of freshly prepared 2M Dithiothreitol in RNase Free Water) to the 1.5 ml microcentrifuge tube.<br>• Use the provided Micropestle to grind the tissue pellet a few times.<br>• Shear tissue by passing lysate through a 20-G needle syringe 10 times then incubate at room temp. for 3 minutes.<br>• Place a Filter Column in a 2 ml Collection Tube and transfer the sample mixture to the Filter Column.<br>• Centrifuge for 30 seconds at 1,000 x g then discard the Filter Column.<br>• Carefully transfer the filtrate to a new 1.5 ml microcentrifuge tube.</td></tr>
<tr><td>Step 2<br>RNA Binding</td><td>• Add 400 µl of 70% ethanol prepared in ddH2O (RNase and DNase-free) and shake the mixture vigorously.<br>NOTE: If precipitate appears, break it up as much as possible with a pipette.<br>• Place a RB Column in a 2 ml Collection tube then transfer the mixture to the RB Column.<br>• Centrifuge at 14-16,000 x g for 1 minute.<br>NOTE: If the lysate mixture could not flow past the RB Column membrane following centrifugation, increase the centrifuge time until the lysate mixture passes completely.<br>• Discard the flow-through then place the RB Column back in the 2 ml Collection Tube.<br>Optional Step 1: In Column DNase I Digestion<br>The amount of DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield.<br>1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000 x g for 30 seconds.<br>2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.<br>3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:
<table>
<tr><td>DNase I</td><td>5 µl (2 U/µl)</td></tr>
<tr><td>DNase I Reaction Buffer</td><td>45 µl</td></tr>
<tr><td>Total Volume</td><td>50 µl</td></tr>
</table>
4. Gently pipette DNase I solution (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RB column matrix.<br>5. Incubate the column for 15 minutes at room temperature (20-30°C) then proceed with Step 3 RNA Wash.</td></tr>
<tr><td>Step 3<br>Wash</td><td>• Add 400 µl of W1 Buffer to the RB Column then centrifuge at 14-16,000 x g for 30 seconds.<br>• Discard the flow-through then place the RB Column back in the 2 ml Collection Tube.<br>• Add 600 µl of Wash Buffer (make sure ethanol was added) into the RB Column.<br>• Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.<br>• Place the RB Column back in the 2 ml Collection Tube.<br>• Add 600 µl of Wash Buffer (make sure ethanol was added) into the RB Column.<br>• Centrifuge at 14-16,000 x g for 30 seconds.<br>• Discard the flow-through then place the RB Column back in the 2 ml Collection Tube.<br>• Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.</td></tr>
</table>

<table>
<tr><td>Step 4<br>RNA Elution</td><td>
• Place the dried RB Column in a clean 1.5 ml microcentrifuge tube.<br>
• Add 50 µl of RNase-free Water to the CENTER of the column matrix.<br>
• Let stand for at least 2 minutes to ensure the RNase-free Water is absorbed by the matrix.<br>
• Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.<br>
Optional Step 2: DNA Digestion In Solution<br>
1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:
<table>
<tr><td>RNA in RNase-free Water</td><td>1 - 40 µl</td></tr>
<tr><td>DNase I</td><td>0.5 µl/µg RNA</td></tr>
<tr><td>DNase I Reaction Buffer</td><td>5 µl</td></tr>
<tr><td>RNase-free Water</td><td>Add to final volume = 50 µl</td></tr>
<tr><td>Total Volume</td><td>50 µl</td></tr>
</table>
2. Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the microcentrifuge tube at 37°C for 15-30 minutes.<br>
3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) then incubate the microcentrifuge tube at 65°C for 10 minutes.<br>
4. Repurify the RNA sample by adding 250 µl of RB Buffer to the 50 µl DNase I reaction mixture then mix well by vortex. Add 300 µl of 70% ethanol then mix well by vortex. Transfer all of sample mixture to a new RB Column. Centrifuge at 14-16,000 x g for 1 minute. Discard the flow through. Proceed with Step 3 RNA Wash.
</td></tr>
</table>

# Troubleshooting

| Problem | Possible Reasons/Solution |
|---|---|
| Clogged Column | • Insufficient disruption and/or homogenization/too much starting material<br>• Centrifugation temperature was too low (should be 20°C to 25°C) |
| Low RNA Yield | • Insufficient disruption and homogenization/too much starting material<br>• RNA still bound to RNA spin column membrane or ethanol carryover |
| RNA Degradation | • Harvested tissue not immediately stabilized/inappropriate handling of starting material or RNase contamination |

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