Total RNA Mini Kit (Tissue)

For research use only

Sample: up to 25 mg of tissue

Yield: 5-30 µg

Format: spin column

Operation Time: within 15 minutes

Elution Volume: 25-100 µl

Storage: dry at room temperature (15-25ºC)

Geneaid

CERTIFICATE NO. QAIC/TW/50077

ISO 9001:2008 QMS

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 Geneaid'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	RT004 RTD004	RT050 RTD050	RT100 RTD100	RT300 RTD300
RB Buffer	2 ml	30 ml	60 ml	130 ml
DNase I[1] (2U/µl) (RTD004/050/100/300 Only)	20 µl	275 µl	550 µl	550 µl x 3
DNase I Reaction Buffer (RTD004/050/100/300 Only)	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

Order Information

Total RNA Purification		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBY050/100/300
miRNA Isolation Kit	50/100 preps	RMI050/100
GENEzol™ Reagent	100/200 rxns	GZR050/100/200
GENEzol™ TriRNA Bacteria Kit	50/100 rxns	GZB050/100
GENEzol™ TriRNA Pure Kit	50/100/200 preps	GZX050/100/200
TriRNA Pure Kit	50/100/200 preps	TRP050/100/200
RNA Pure Kit	50/100 preps	PR050/100
GENEzol™ 96 Well TriRNA Pure Kit	4/10 x 96 preps	96GZX04/10

[1]DNase I is shipped at room temperature and should be stored at -20ºC 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. The additional **Wash Buffer x 12.5 ml** is **only** included in **RTD100**.

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, ddH_2O (RNase/DNase-free) to prepare 70% ethanol, microcentrifuge tubes, pipette tips, ß-mercaptoethanol

Total RNA Mini Kit (Tissue) Protocol

Sample Prep.

Fresh or Frozen Animal Tissue

- Cut off up to 25 mg of fresh/frozen tissue and transfer to a 1.5 ml microcentrifuge tube then proceed with Cell Lysis.

NOTE: If using frozen animal tissue, the sample must have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use to avoid RNA degradation.

Step 1 Cell Lysis

- 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.
- Use the provided **Micropestle** to grind the tissue a few times.
- Shear tissue by passing lysate through a 20-G needle syringe 10 times then incubate at room temp. for 3 minutes.
- Place a **Filter Column** in a **2 ml Collection Tube** and transfer the sample mixture to the **Filter Column**.
- Centrifuge for 30 seconds at 1,000 x g then discard the **Filter Column**.
- Carefully transfer the filtrate to a new 1.5 ml microcentrifuge tube.

Step 2 RNA Binding

- Add **400 µl of 70% ethanol prepared in ddH_2O (RNase and DNase-free)** and shake the mixture vigorously.

NOTE: If precipitate appears, break it up as much as possible with a pipette.

- Place a **RB Column** in a **2 ml Collection tube** then transfer the mixture to the **RB Column**.
- Centrifuge at 14-16,000 x g for 1 minute.

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.

- Discard the flow-through then place the **RB Column** back in the **2 ml Collection Tube**.

Optional Step 1: In Column DNase I Digestion

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.

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.
2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.
3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total Volume	50 µl

4. Gently pipette DNase I solution (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RB column matrix.
5. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with Step 3 RNA Wash.

Step 3 Wash

- Add **400 µl of W1 Buffer** to the **RB Column** then centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through then place the **RB Column** back in the **2 ml Collection Tube**.
- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column**.
- Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
- Place the **RB Column** back in the **2 ml Collection Tube**.
- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column**.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through then place the **RB Column** back in the **2 ml Collection Tube**.
- Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

Step 4 RNA Elution

- Place the dried **RB Column** in a clean 1.5 ml microcentrifuge tube.
- Add **50 µl of RNase-free Water** to the CENTER of the column matrix.
- Let stand for at least 2 minutes to ensure the **RNase-free Water** is absorbed by the matrix.
- Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

Optional Step 2: DNA Digestion In Solution

1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2. Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.
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.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the Geneaid™ RNA Cleanup Kit instead of stopping the reaction with EGTA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	• Insufficient disruption and/or homogenization/too much starting material • Centrifugation temperature was too low (should be 20ºC to 25ºC)
Low RNA Yield	• Insufficient disruption and homogenization/too much starting material • 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