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IBI SCIENTIFIC

INSTRUCTION MANUAL

Total RNA Mini Kit (Blood/Cultured Cell)

IB47320 (4 prep sample kit)
IB47321 (50 prep kit)
IB47322 (100 prep kit)
IB47323 (300 prep kit)

Advantages

Sample: up to 300 µl of whole blood, up to 5 x 10^6 cultured animal cells

Yield: 2-3 µg (300 µl blood), 25-30 µg (1 x 10^6 293T cells)

Format: spin column

Operation Time: within 20 minutes

Elution Volume: 25-100 µl

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

Introduction

The Total RNA Mini Kit (Blood/Cultured Cell) was designed specifically for purifying total RNA from fresh whole human blood and cultured cells. 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 and once any contaminants have been removed, using the Wash Buffer, the purified total RNA is eluted by RNase-free Water. High quality total RNA can be purified in less than 20 minutes without phenol extraction or alcohol precipitation. The purified RNA is ready for use in RT-PCR, northern blotting, primer extension, mRNA selection and cDNA synthesis.

Quality Control

The quality of the Total RNA Mini Kit (Blood/Cultured Cell) is tested on a lot-to-lot basis according to IBI's ISO-certified quality management system. Total RNA is isolated from 300 µl of whole blood, quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

Component	IB47320	IB47321	IB47322	IB47323
RBC Lysis Buffer	10 ml	100 ml	200 ml	500 ml
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
RB Columns	4	50	100	300
2 ml Collection Tubes	8	100	200	600

[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
RNA Pure Kit	50/100 rxns	IB47641/642

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 Wash Buffer prior to initial use
- Additional Requirements: phosphate-buffered saline (PBS), absolute ethanol, ddH2O (RNase/DNase-free), microcentrifuge tubes, pipette tips, ß-mercaptoethanol, 0.10-0.25% Trypsin

Total RNA Mini Kit (Tissue) Protocol

Step 1 Cell Harvesting and Lysis

Fresh Human Blood

- Collect fresh human blood in anticoagulant-treated collection tubes.
- Add 1 ml of RBC Lysis Buffer and 300 µl of whole human blood to a sterile 1.5 ml microcentrifuge tube. Mix by inversion.
- Incubate the tube on ice for 10 minutes (briefly vortex twice during incubation).
- Centrifuge at 3,000 x g for 5 minutes then remove the supernatant completely.
- Add 400 µl of RB Buffer and 4 µl of ß-mercaptoethanol (or 8 µl of freshly prepared 2M Dithiothreitol in RNase-free Water).
- Resuspend the cells by pipetting then incubate at room temperature for 5 minutes.

Adherent Cultured Animal Cells

A. Cell lysis in a culture dish

- Aspirate the culture medium completely.
- Add 400 µl of RB Buffer and 4 µl of ß-mercaptoethanol (or 8 µl of freshly prepared 2M Dithiothreitol in RNase-free Water) immediately to the culture dish (up to 5 x10^6 cells).
- Incubate at room temperature for 5 minutes then transfer the cell lysate to a 1.5 ml microcentrifuge tube.

B. Trypsinize cell prior to cell lysis

- Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS.
- Once cells have detached, add the medium and transfer to a 15 ml centrifuge tube.
- Proceed with Suspension Cultured Animal Cells.

Suspension Cultured Animal Cells

- Transfer cells (up to 5 x 10^6) to a 1.5 ml microcentrifuge tube or 15 ml centrifuge tube.
- Harvest by centrifugation for 5 minutes at 300 x g then remove the supernatant.
- Add 400 µl of RB Buffer and 4 µl of ß-mercaptoethanol (or 8 µl of freshly prepared 2M Dithiothreitol in RNase Free Water).
- Resuspend the cells by pipetting then incubate at room temperature for 5 minutes.

Step 2 RNA Binding

- Add 400 µl of 70% ethanol prepared in ddH2O (RNase and DNase-free).
- Shake the mixture vigorously. Break up any precipitate as much as possible by pipetting.
- Place a RB Column in a 2 ml Collection Tube and transfer 500 µl of the mixture to the RB Column.
- Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through.
- Transfer the remaining mixture to the same RB Column then centrifuge at 14-16,000 x g for 1 minute.
- Discard the flow-through and place the RB Column in a new 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 to mix (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 (20-30°C) then proceed with Step 3 RNA Wash.

<table>
<tr><td>Step 3
Wash</td><td>• Add 400 µl of W1 Buffer into 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 then discard the flow-through.
• Place the RB Column back in the 2 ml Collection Tube and centrifuge at 14-16,000 x g for 3 min. to dry the column matrix.</td></tr>
<tr><td>Step 4
RNA Elution</td><td>• Place the dried RB Column in a clean 1.5 ml microcentrifuge tube.
• Add 50 µl of RNase-free Water into the CENTER of the column matrix.
• Let stand for at least 1 minute to ensure the RNase-free Water is absorbed.
• 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:
<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 to mix (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 RNA Pure Kit instead of stopping the reaction with EGTA.</td></tr>
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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	• RNA still bound to the RB Column membrane • Ethanol carryover
RNA Degradation	• Harvested sample not immediately stabilized/inappropriate handling of starting material • RNase contamination

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