

Total RNA Mini Kit (Blood/Cultured Cell)

For research use only

Sample	: up to 300 µl of whole blood, cultured animal cells (up to 5×10^6), cultured bacterial cells (up to 1×10^9) and fungus cells (up to 5×10^7)
Yield	: 5-30 µg
Format	: spin column
Operation time	: within 60 minutes

Geneaid



www.geneaid.com

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 (optional DNase treatments can be followed to remove unwanted DNA residue). RNA in the chaotropic salt is bound by the glass fiber matrix of the spin column (1) and once any contaminants have been removed, using the Wash Buffer (containing ethanol), the purified total RNA is eluted by RNase-free water. The entire procedure can be completed within 1 hour and 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 by isolating total RNA from blood and cultured cell samples. The purified RNA is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	RB004	RB050	RB100	RB300
RBC Lysis Buffer	10 ml	100 ml	200 ml	500 ml
RB Buffer	2 ml	30 ml	60 ml	130 ml
RT Buffer	1.5 ml	15 ml	30 ml	75 ml
W1 Buffer	2 ml	30 ml	50 ml	130 ml
Wash Buffer ¹	1 ml	12.5 ml	25 ml	50 ml x 2
(Add Ethanol)	(4 ml)	(50 ml)	(100 ml)	(200 ml) x 2
RNase-free water	1 ml	6 ml	6 ml	30 ml
RB Column	4 pcs	50 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs

Order Information

Product Name	Package size	Cat. No.
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	RBM10/25
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Maxi Kit (Tissue)	10/25 preps	RTM10/25
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Total RNA Maxi Kit (Plant)	10/25 preps	RPM10/25
miRNA Isolation Kit	100 preps	PU009100
96-Well Total RNA Kit	2/4/10 x 96 Wells	RBP02/04/10

¹Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Caution

RB Buffer contains chaotropic salt which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Total RNA Mini Kit (Blood/Cultured Cell) Protocol

- Add absolute ethanol to Wash Buffer prior to initial use (see the bottle label for volume).
- Additional Requirements: PBS (phosphate-buffered saline), absolute ethanol, microcentrifuge tubes (RNase free), β -mercaptoethanol

DNA Residue Degradation options:

► Optional Step 1 (DNA Residue Degradation): Add 100 μ l of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 50 μ g/ml BSA at 25°C} to the center of the RB Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

► Optional Step 2 (DNA Residue Degradation): Add 2 μ l of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 50 μ g/ml BSA at 25°C} to the final elution sample. Let stand for 10 minutes at room temperature.

Step 1 RBC Lysis/ Cell Harvesting	<p>Fresh human blood</p> <ul style="list-style-type: none"> ● Collect fresh human blood in anticoagulant-treated collection tubes. ● Add 1 ml of RBC Lysis Buffer to a sterile 1.5 ml microcentrifuge tube. ● Add 300 μl of whole human blood and mix by inversion. ● Incubate the tube on ice for 10 minutes (briefly vortex twice during incubation). ● Centrifuge for 5 minutes at 3,000 x g at 4°C. ● Remove the supernatant completely and resuspend the cells in 100 μl of RBC Lysis Buffer by pipetting the pellet. <p>Cultured animal cells</p> <p>If using adherent cells, trypsinize the cells before harvesting.</p> <ul style="list-style-type: none"> ● Transfer the cells (up to 5×10^6) to a 1.5 ml microcentrifuge tube and harvest by centrifugation for 20 seconds at 6,000 x g. ● Remove the supernatant completely and resuspend the cells in 100 μl of PBS or RBC Lysis Buffer.
Step 2 Cell Lysis	<ul style="list-style-type: none"> ● Add 400 μl of RB Buffer and 4 μl of β-mercaptoethanol to the resuspended cells from Step 1 and shake vigorously. ● Incubate at room temperature for 5 minutes.
Step 3 RNA Binding	<ul style="list-style-type: none"> ● Add 500 μl of 70% ethanol to the sample lysate from Step 2 and shake vigorously (break up any precipitate with pipetting). ● Place a RB Column in a 2 ml Collection Tube. ● Transfer 500 μl of the ethanol-added mixture to the RB Column. ● Centrifuge at 14-16,000 x g for 1 minute. ● Discard the flow-through and transfer the remaining mixture to the same RB Column. ● 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. <p>Optional Step 1: DNA Residue Degradation (see options above)</p>
Step 4 Wash	<ul style="list-style-type: none"> ● Add 400 μl of W1 Buffer into the RB Column. ● Centrifuge at 14-16,000 x g for 1 minute. ● Discard the flow-through and place the RB Column back in the 2 ml Collection Tube. ● Add 600 μl of Wash Buffer (ethanol added) into the RB Column. ● Centrifuge at 14-16,000 x g for 1 minute. ● Discard the flow-through and place the RB Column back in the 2 ml Collection Tube. ● Add 600 μl of Wash Buffer (ethanol added) into the RB Column. ● Centrifuge at 14-16,000 x g for 1 minute. ● Discard the flow-through and 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 5 RNA Elution	<ul style="list-style-type: none"> ● Place the dried RB Column in a clean 1.5 ml microcentrifuge tube (RNase-free). ● Add 50 μl of RNase-free water into the center of the column matrix. ● Let stand for 3 minutes or until the water has been absorbed by the matrix. ● Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA. <p>Optional Step 2: DNA Residue Degradation (see options above)</p>

Total RNA Mini Kit (Blood/Cultured Cell) Bacteria Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: absolute ethanol, microcentrifuge tubes (RNase free), β -mercaptoethanol
- Gram-positive bacteria: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0), prepare the lysozyme buffer immediately prior to use

DNA Residue Degradation options:

► Optional Step 1 (DNA Residue Degradation): Add 100 μ l of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 50 μ g/ml BSA at 25°C} to the center of the RB Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

► Optional Step 2 (DNA Residue Degradation): Add 2 μ l of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 50 μ g/ml BSA at 25°C} to the final elution sample. Let stand for 10 minutes at room temperature.

Step 1 Cell Harvesting	<p>Gram-negative bacteria</p> <ul style="list-style-type: none"> ☛ Transfer the bacterial culture (up to 1×10^9) to a 1.5 ml microcentrifuge tube. ☛ Centrifuge for 1 minute at 14-16,000 x g and remove the supernatant completely. ☛ Vortex the cell pellet for 30 seconds. ☛ Add 200 μl of RT Buffer to the tube and resuspend the cell pellet by vortex or pipetting. ☛ Incubate at room temperature for 5 minutes. <p>Gram-positive bacteria</p> <ul style="list-style-type: none"> ☛ Transfer the bacterial culture (up to 1×10^9) to a 1.5 ml microcentrifuge tube. ☛ Centrifuge for 1 minute at 14-16,000 x g and remove the supernatant completely. ☛ Add 200 μl of lysozyme buffer to the tube and resuspend the cell pellet by vortex or pipetting. ☛ Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.
Step 2 Cell Lysis	<ul style="list-style-type: none"> ☛ Add 300 μl of RB Buffer and 3 μl β-mercaptoethanol to the sample lysate from Step 1 and mix by vortex. ☛ Incubate at room temperature for 5 minutes. ☛ Centrifuge at 14-16,000 x g for 2 minutes. ☛ Transfer the supernatant to a new 1.5 ml microcentrifuge tube.
Step 3 RNA Binding	<ul style="list-style-type: none"> ☛ Add 500 μl of 70% ethanol to the sample lysate from Step 2; mix immediately by pipetting. ☛ Place a RB Column in a 2 ml Collection Tube. ☛ Transfer 500 μl of the ethanol-added mixture to the RB Column. ☛ Centrifuge at 14-16,000 x g for 1 minute. ☛ Discard the flow-through and transfer the remaining mixture to the same RB Column. ☛ 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. <p>Optional Step 1: DNA Residue Degradation (see options above)</p>
Step 4 Wash	<ul style="list-style-type: none"> ☛ Add 400 μl of W1 Buffer to the RB Column. ☛ Centrifuge at 14-16,000 x g for 1 minute. ☛ Discard the flow-through and place the RB Column back in the 2 ml Collection Tube. ☛ Add 600 μl of Wash Buffer (ethanol added) into the RB Column. ☛ Centrifuge at 14-16,000 x g for 1 minute. ☛ Discard the flow-through and place the RB Column back in the 2 ml Collection Tube. ☛ Add 600 μl of Wash Buffer (ethanol added) into the RB Column. ☛ Centrifuge at 14-16,000 x g for 1 minute. ☛ Discard the flow-through and 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 5 RNA Elution	<ul style="list-style-type: none"> ☛ Place the dried RB Column in a clean 1.5 ml microcentrifuge tube (RNase-free). ☛ Add 50 μl of RNase-free water into the center of the column matrix. ☛ Let stand for 3 minutes or until the water is absorbed by the matrix. ☛ Centrifuge at 14-16,000 x g for 1 minute to elute purified RNA. <p>Optional Step 2: DNA Residue Degradation (see options above)</p>

Total RNA Mini Kit (Blood/Cultured Cell) Fungus Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol; 10 mM CaCl₂; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

DNA Residue Degradation options:

► Optional Step 1 (DNA Residue Degradation): Add 100 µl of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 50 µg/ml BSA at 25°C} to the center of the RB Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

► Optional Step 2 (DNA Residue Degradation): Add 2 µl of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 50 µg/ml BSA at 25°C} to the final elution sample. Let stand for 10 minutes at room temperature.

Step 1 Cell Harvesting	<ul style="list-style-type: none"> ● Harvest fungus cells (up to 5×10^7) by centrifugation for 10 minutes at 5,000 x g. ● Discard the supernatant and resuspend the pellet in 600 µl of sorbitol buffer. ● Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 minutes. ● Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. ● Remove the supernatant and proceed with Lysis Step of the Bacteria Protocol.
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Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	<ul style="list-style-type: none"> ● Inefficient disruption and/or homogenization ● Too much starting material ● Centrifugation temperature was too low (should be 20-25°C)
Low RNA Yield	<ul style="list-style-type: none"> ● Insufficient disruption and homogenization ● Too much starting material ● RNA still bound to the RB Column membrane ● Ethanol carryover
RNA Degradation	<ul style="list-style-type: none"> ● Harvested sample not immediately stabilized ● Inappropriate handling of starting material ● RNase contamination