Total RNA Maxi Kit (Blood/Cultured Cell)

For research use only

Sample : up to 5 ml of whole human blood, cultured animal cells (up to 1 x 10^8), cultured bacterial cells (up to 1 x 10^{10})

Yield : 50-300 µg

Format : spin column

Operation time : within 60 minutes

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Introduction

The Total RNA Maxi 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 and optional DNase treatments can be followed to remove unwanted DNA residue. The RNA in the chaotropic salt is bound by the glass fiber matrix of the spin column (1). 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 and cDNA Library Construction.

Quality Control

The quality of the Total RNA Maxi 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	RBM02	RBM10	RBM25
RBC Lysis Buffer	40 ml	175 ml	135 ml x 3
RB Buffer	12 ml	60 ml	130 ml
RT Buffer	6 ml	30 ml	60 ml
W1 Buffer	10 ml	50 ml	130 ml
Wash Buffer[1] (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	50 ml (200 ml) 12.5 ml (50 ml)
RNase-free water	1 ml	6 ml	30 ml
RB Maxi Column	2 pcs	10 pcs	25 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

[1]Add 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 Maxi Kit (Blood/Cultured Cell) Blood Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: absolute ethanol, centrifuge tubes (RNase-free), ß-mercaptoethanol, DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC}

Step 1 RBC Lysis/ Cell Harvesting	**Fresh human blood** • Collect fresh human blood in anticoagulant-treated collection tubes. • Add 3 ml of whole blood to a sterile 15 ml centrifuge tube. • Add 3 x the sample volume of **RBC Lysis Buffer** 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. • Remove the supernatant completely and resuspend the cells in **200 µl of RBC Lysis Buffer** by flicking the tube.
Step 2 Cell Lysis	• Add **5 ml of RB Buffer** and 50 µl of ß-mercaptoethanol to the resuspended cells from Step 1 and shake vigorously (break up any precipitate with pipetting). • Incubate at room temperature for 5 minutes. **Optional Step: DNA Residue Degradation** Add 10 µl DNase I (2 U/µl) to the 15 ml centrifuge tube. Let stand for 5 minutes at room temperature. Proceed to Step 3 RNA Binding.
Step 3 RNA Binding	• Add 2.5 ml of absolute ethanol to the sample lysate from Step 2 and shake vigorously (break up any precipitate with pipetting). • Place a **RB Maxi Column** in a 50 ml centrifuge tube. • Transfer up to 10 ml of the ethanol-added mixture to the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 5 minutes. • Discard the flow-through and add the remaining mixture to the same **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 5 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube.
Step 4 Wash	• Add **4 ml of W1 Buffer** into the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. • Add **6 ml of Wash Buffer** (ethanol added) into the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. • Add **6 ml of Wash Buffer** (ethanol added) into the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 5 RNA Elution	• Place the dried **RB Maxi Column** in a clean 50 ml centrifuge tube (RNase-free). • Add **500 µl of RNase-free water** into the center of the column matrix. • Let stand for 5 minutes or until the water has been absorbed by the matrix. • Centrifuge at 14-16,000 x g for 5 minutes to elute the purified RNA.

Total RNA Maxi Kit (Blood/Cultured Cell) Cultured Cell Protocol

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional Requirements: PBS (phosphate-buffered saline), absolute ethanol, centrifuge tubes (RNase-free), ß-mercaptoethanol, DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC}

Step 1 Cell Harvesting	**Suspension cultured animal cells** • Transfer the cells (up to 1 x 10^8) to a 15 ml centrifuge tube and harvest with centrifugation for 5 minutes at 300 x g. • Remove the supernatant completely and resuspend the cells in 200 µl of PBS or RBC Lysis Buffer. • Proceed with the Lysis Step (Step 2) of the Blood Protocol.
	Adherent cultured cells If using adherent cultured cells, trypsinize cells before lysis or lyse cells directly in a culture dish. • To trypsinize cells, remove the medium and wash cells with PBS. • Aspirate PBS and add 0.10–0.25% Trypsin in PBS to trypsinize the cells. • Once the cells have detached, add the medium and transfer them to a 15 ml centrifuge tube. • Pellet cells as suspension cultured animal cells. • Proceed with the Lysis Step (Step 2) of the Blood Protocol.
	To lyse cells in a culture dish or flask • Remove the culture medium. • Add **5 ml of RB Buffer** and 50 µl of ß-mercaptoethanol to a culture dish or flask. • Let the **RB Buffer** cover the dish or flask by shaking for 5 minutes. • Collect the cell lysate with a rubber policeman and transfer it to a 15 ml centrifuge tube. **Optional Step: DNA Residue Degradation** • Add 10 µl of DNase I (2 U/µl) to the 15 ml centrifuge tube. • Let stand for 5 minutes at room temperature. • Centrifuge at 14-16,000 x g for 3 minutes. • Transfer the supernatant to a new 15 ml centrifuge tube. • Proceed with the Binding Step (Step 3) of the Blood Protocol.

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

- Additional requirements: ß-mercaptoethanol, centrifuge tubes (RNase-free), DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5),10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC}
- 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**

Step 1 Cell Harvesting	**Gram-negative bacteria** • Transfer the bacterial culture (up to 1 x 10^{10}) to a 15 ml centrifuge tube. • Centrifuge for 5 minutes at 14-16,000 x g and remove the supernatant completely. • Vortex the cell pellet for 30 seconds. • Add **2 ml of RT Buffer** to the tube and resuspend the cell pellet by vortex or pipetting. • Incubate at room temperature for 5 minutes. **Gram-positive bacteria** • Transfer the bacterial culture (up to 1 x 10^{10}) to a 15 ml centrifuge tube. • Centrifuge for 5 minutes at 14-16,000 x g and remove the supernatant completely. • Add 2 ml 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	• Add **3 ml of RB Buffer** and 30 µl of ß-mercaptoethanol to the sample lysate from Step 1 and mix by vortex. • Incubate at room temperature for 5 minutes.
	Optional Step: DNA residue degradation Add 10 µl DNase I (2 U/µl) to the 15 ml centrifuge tube. • Let stand for 5 minutes at room temperature.
	• Centrifuge at 14-16,000 x g for 3 minutes. • Transfer the supernatant to a new 15 ml centrifuge tube. • Proceed to Step 3 RNA Binding.
Step 3 RNA Binding	• Add 2.5 ml of absolute ethanol to the sample lysate from Step 2 and mix immediately by pipetting. • Place a **RB Maxi Column** in a 50 ml centrifuge tube. • Add up to 10 ml of the ethanol-added mixture to the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 5 minutes. • Discard the flow-through and add the remaining mixture to the same **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 5 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube.
Step 4 Wash	• Add **4 ml of W1 Buffer** into the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. • Add **6 ml of Wash Buffer** (ethanol added) into the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. • Add **6 ml of Wash Buffer** (ethanol added) into the **RB Maxi Column**. • Centrifuge at 14-16,000 x g for 3 minutes. • Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 5 RNA Elution	• Place the dried **RB Maxi Column** in a clean 50 ml centrifuge tube (RNase-free). • Add **500 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 5 minutes to elute the purified RNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged RB Maxi Column	• Inefficient disruption and/or homogenization • Too much starting material • Centrifugation temperature was too low (should be 20-25ºC)
Low RNA Yield	• Insufficient disruption and homogenization • Too much starting material • RNA still bound to RB Maxi Column membrane • Ethanol carryover
RNA Degradation	• Harvested sample not immediately stabilized • Inappropriate handling of starting material • RNase contamination