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HiYield Total RNA Maxi Kit (Blood/Bacteria/Cultured Cells)

Cat. No.:	YRBM10	YRBM25
Product Name:	HiYield Total RNA Maxi Kit (Blood/Bacteria/Cultured Cells)	
Reactions:	10	25
Sample:	Up to 5 ml of whole blood, 10^8 of cultured animal cells, 10^{10} of cultured bacterial cells	
Yield:	Up to 300 ug	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 60 Minutes	

Introduction

HiYield Total RNA Maxi Kit (Blood/Bacteria/Cultured Cells) is designed specifically for purifying total RNA from fresh human whole blood and cultured cells. The entire procedure can be completed within 1 hour and the purified RNA is ready for direct use in RT-PCR, Northern Blotting, Primer Extension, mRNA Selection and cDNA Synthesis.

Features

Ready-to-use RNA for many downstream applications.
Excellent Recovery of RNA.
Complete removal of contaminants and inhibitors.

Components

ITEM	YRBM10	YRBM25
RBC Lysis Buffer	175ml	405ml
RB Buffer	60ml	130ml
RT Buffer	30ml	60ml
W1 Buffer	50ml	130ml
Wash Buffer*	25ml	62.5ml
RNase-Free Water	6ml	30ml
RBM Column	10pcs	25pcs

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

Applications

Purified RNA is ready for direct use in RT-PCR, Real-Time RT-PCR, Northern Blotting, Primer Extension, RNase Protection Assays, mRNA Selection, cDNA Synthesis.

Quality Control

The quality of HiYield Total RNA Maxi Kit (Blood/Bacteria/Cultured Cells) is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 3 ml of fresh human whole blood. More than 10 ug of total RNA was quantified with a spectrophotometer and checked by formaldhyde agarose gel analysis. Finally, RT-PCR was used to ensure the quality of total RNA.

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.

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Fresh Human Whole Blood Protocol

Things to do before starting

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	Procedure
Step 1 RBC Lysis / Cell Harvesting	★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 500 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.
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 **RBM Column** in a 50 ml centrifuge tube. ★Transfer up to 10 ml of the ethanol-added mixture to the **RBMColumn**. ★Centrifuge at full speed for 5 minutes. ★Discard the flow-through and add the remaining mixture to the same **RBM Column**. ★Centrifuge at full speed for 5 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube.

Step 4 **Wash**	★Add **4 ml of W1 Buffer** into the **RBM Column**. ★Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) into the **RBM Column**. ★Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) into the **RBM Column**. ★Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube. ★Centrifuge at full speed for 3 minutes to dry the column matrix.
Step 5 **RNA** **Elution**	★Place the dried **RBM 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 full speed for 5 minutes to elute the purified RNA.

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Cultured Cells Protocol

Things to do before starting

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	Procedure
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 Fresh whole 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 Fresh Whole 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 full speed for 3 minutes. ★Transfer the supernatant to a new 15 ml centrifuge tube. ★Proceed with the Binding Step (Step 3) of the Fresh Whole Blood Protocol.

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Bacteria Protocol

Things to do before starting

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

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 he lysozyme buffer immediately prior to use.**

Step	Procedure
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 full speed 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 full speed 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 full speed 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 **RBM Column** in a 50 ml centrifuge tube. ★Add up to 10 ml of the ethanol-added mixture to the **RBM Column**. ★Centrifuge at full speed for 5 minutes. ★Discard the flow-through and add the remaining mixture to the same **RBM Column**. ★Centrifuge at full speed for 5 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube.
Step 4 **Wash**	★Add **4 ml of W1 Buffer** into the **RBM Column**. ★Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) into the **RBM Column**. ★Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) into the **RBM Column**. ★Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RBM Column** back in the 50 ml centrifuge tube. ★Centrifuge at full speed for 3 minutes to dry the column matrix.
Step 5 **RNA Elution**	★Place the dried **RBM 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 full speed for 5 minutes to elute the purified RNA.

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Troubleshooting

Problem	Possible Reasons/Solution
Clogged RBM 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