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

Cat. No.:	YRB50	YRB100	YRB300
Product Name:	HiYield Total RNA Mini Kit (Blood/Bacteria/Cultured Cells)		
Reactions:	50	100	300
Sample:	Up to 300 ul of whole blood, 5 x 10^6 of cultured animal cells, 10^9 of cultured bacterial cells and 5 x 10^7 of fungus cells		
Yield:	Up to 30 ug		
Format:	Spin Column		
Operation:	Centrifuge		
Operation Time:	Within 30 Minutes		

Introduction

HiYield Total RNA Mini 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	YRB50	YRB100	YRB300
RBC Lysis Buffer	100ml	200ml	500ml
RB Buffer	30ml	60ml	130ml
RT Buffer	15ml	30ml	75ml
W1 Buffer	30ml	50ml	130ml
Wash Buffer*	12.5ml	25ml	100ml
RNase-Free Water	6ml	6ml	30ml
RB Column	50pcs	100pcs	300pcs
2 ml Collection Tube	100 pcs	200 pcs	600pcs

*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 Mini Kit (Blood/Bacteria/Cultured Cells) is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 300 ul of fresh human whole blood. More than 1 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.

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, microcentrifuge 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	★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 1,000 x g at 4ºC. ★Remove the supernatant completely and resuspend the cells in **100 µl of RBC Lysis Buffer** by pipetting the pellet.
Step 2 Cell Lysis	★Add **400 ul of RB Buffer** and 4 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.
Step 3 RNA Binding	★Add 500 ul of 70% absolute 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 ull of the ethanol-added mixture to the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and transfer the remaining mixture to the same **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** in a new **2 ml Collection Tube**.
	Optional Step: DNA Residue Degradation ★Add 100 µl DNase I (2 U/µl) to the center of the RB Column matrix. ★Let stand for 10 minutes at room temperature.

Step 4 Wash	★Add **400 ul of W1 Buffer** into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Centrifuge at full speed for 3 minutes to dry the column matrix.
Step 5 RNA Elution	★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 full speed for 1 minute to elute the purified RNA.
	Optional Step: DNA Residue Degradation ★Add 2 µl DNase I (2 U/µl) to the final elution sample. ★Let stand for 10 minutes at room temperature.

Cultured Animal 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, microcentrifuge 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	★If using adherent cells, trypsinize the cells before harvesting. ★Transfer the cells (up to 5 x 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	★Add **400 ul of RB Buffer** and 4 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.
Step 3 RNA Binding	★Add 500 ul of 70% absolute 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 ull of the ethanol-added mixture to the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and transfer the remaining mixture to the same **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** in a new **2 ml Collection Tube**.
	Optional Step: DNA Residue Degradation ★Add 100 µl DNase I (2 U/µl) to the center of the RB Column matrix. ★Let stand for 10 minutes at room temperature.

Step 4 Wash	★Add **400 ul of W1 Buffer** into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Centrifuge at full speed for 3 minutes to dry the column matrix.
Step 5 RNA Elution	★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 full speed for 1 minute to elute the purified RNA.
	Optional Step: DNA Residue Degradation ★Add 2 µl DNase I (2 U/µl) to the final elution sample. ★Let stand for 10 minutes at room temperature.

Cultured Bacterial 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: ß-mercaptoethanol, microcentrifuge 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 1 Cell Harvesting	**Gram-negative bacteria** ★Transfer the bacterial culture (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube. ★Centrifuge for 1 minute at full speed and remove the supernatant completely. ★Vortex the cell pellet for 30 seconds. ★Add **200 ul 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 10^9) to a 1.5 ml microcentrifuge tube. ★Centrifuge for 1 minute at full speed and remove the supernatant completely. ★Add 200 ul 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 **300 ul of RB Buffer** and 3 µl of ß-mercaptoethanol to the sample lysate from Step 1 and mix by vortex. ★Incubate at room temperature for 5 minutes. ★Centrifuge at full speed for 2 minutes. ★Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 **RNA Binding**	★Add 500 ul of 70% absolute 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 ull of the ethanol-added mixture to the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and transfer the remaining mixture to the same **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** in a new **2 ml Collection Tube**.
	Optional Step: DNA Residue Degradation ★Add 100 µl DNase I (2 U/µl) to the center of the RB Column matrix. ★Let stand for 10 minutes at room temperature.
Step 4 **Wash**	★Add **400 ul of W1 Buffer** into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Centrifuge at full speed for 3 minutes to dry the column matrix.
Step 5 **RNA Elution**	★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 full speed for 1 minute to elute the purified RNA.
	Optional Step: DNA Residue Degradation ★Add 2 µl DNase I (2 U/µl) to the final elution sample. ★Let stand for 10 minutes at room temperature.

Fungus 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: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM CaCl2; 0.1 M Tris-HCl pH 7.5; 35mM mercaptoethanol), microcentrifuge 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}

Step 1 Cell Harvesting	★Harvest fungus cells (up to 5 x 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 30ºC for 30 minutes. ★Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. ★Remove the supernatant
Step 2 Cell Lysis	★Add **300 ul of RB Buffer** and 3 µl of ß-mercaptoethanol to the sample lysate from Step 1 and mix by vortex. ★Incubate at room temperature for 5 minutes. ★Centrifuge at full speed for 2 minutes. ★Transfer the supernatant to a new 1.5 ml microcentrifuge tube.
Step 3 RNA Binding	★Add 500 ul of 70% absolute 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 ull of the ethanol-added mixture to the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and transfer the remaining mixture to the same **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** in a new **2 ml Collection Tube**.
	Optional Step: DNA Residue Degradation ★Add 100 µl DNase I (2 U/µl) to the center of the RB Column matrix. ★Let stand for 10 minutes at room temperature.

Step 4 **Wash**	★Add **400 ul of W1 Buffer** into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Add **600 ul of Wash Buffer** (ethanol added) into the **RB Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. ★Centrifuge at full speed for 3 minutes to dry the column matrix.
Step 5 **RNA Elution**	★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 full speed for 1 minute to elute the purified RNA.
	Optional Step: DNA Residue Degradation ★Add 2 µl DNase I (2 U/µl) to the final elution sample. ★Let stand for 10 minutes at room temperature.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged RB 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 Column membrane ★Ethanol carryover
RNA Degradation	★Harvested sample not immediately stabilized ★Inappropriate handling of starting material ★RNase contamination