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HiYield™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue)

Cat. No.:	YTR100	YTR500
Product Name:	HiYield™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue)	
Reactions:	100	500
Sample:	Fresh blood or serum, cultured bacterial cells, cultured animal cells and tissue.	
Yield:	Up to 60 µg for 50 mg of Tissue Up to 30 µg for 3 ml of Cultured Bacterial Cells Up to 30 µg for 5 x 10^6 Cultured Animal Cells	
Format:	Reagent	
Operation:	Centrifuge	
Operation Time:	Within 60 Minutes	

Introduction

HiYield™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue) enables 3-steps operations to isolate total RNA from tissue, cultured animal cells, cultured bacterial cells, blood and serum. The scalable purification procedure gently removes contaminants and inhibitors and allows large-volume samples to be purified for use as long-term references. The unique TR Buffer system ensures total RNA with high yield and good quality from samples of unlimited size. If a larger sample is required, the buffer volume can be scaled proportionately.

DNA phenol extraction is not required. The entire procedure can be completed in 1 hour. RNA purified using HiYield™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue) is highly stable and suited for use in a wide range of applications, such as: RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

Features

1. Convenient, scalable purification procedure
2. Reproducible recoveries for constant results.
3. Ready-to use RNA within 60 minutes.

Contents

ITEM	YTR100	YTR500
TR1 Buffer*	100ml	500ml
TR2 Buffer*	15ml	75ml

* If there is sediment formed in the buffer, incubate at 65℃ for 10 minutes to dissolve it.

Applications

Purified RNA is ready for direct use in RT-PCR, Real-Time PCR, Northern Blot Analysis, mRNA Selection, Microarrays, cDNA synthesis.

Quality Control

The quality of HiYield™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue) is tested on a lot-to-lot basis by isolating total RNA from 50 mg tissue samples. A minimum of 20 µg of total RNA is quantified with a spectrophotometer and checked by electrophoresis.

Caution

Buffers contain irritant agents. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Protocol

Additional Requirements:

RNase-free microcentrifuge tubes, absolute ethanol for preparing 70% ethanol in RNase-free water, Chloroform, Isopropanol, mortar and pestle, ß-mercaptoethanol, RNase-free water, RBC Lysis Buffer (fresh blood samples only).

Notes:

If a larger sample volume is required, scale the TR1 Buffer proportionately. For complete DNA Degradation, add 2µl 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 25ºC} to the final sample in the RNA Precipitation Step. Let stand for 10 minutes at room temperature.

Sample Preparation Tissue Dissociation or Cultured Cell Harvesting or RBC Lysis	**Tissue** ★Cut off 50 mg of fresh tissue. ★Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.
	Cultured Animal/Bacterial Cells ★Transfer 5 x 10^6 cultured animal cells or 1.5 ml of cultured bacterial cells to a microcentrifuge tube. ★Microcentrifuge at 13,000rpm for 1 minute and remove the supernatant completely (If more than 1.5 ml of bacterial culture is used, repeat this step). ★Apply 20 µl of RNase-free water to resuspend the pellet.
	Fresh Blood/Frozen Blood ★Collect fresh blood/frozen blood in EDTA-NA2 treated collection tubes (or other anticoagulant mixtures). ★Transfer up to 300 µl of fresh blood/frozen blood to a 1.5 ml microcentrifuge tube. (If the blood sample is more than 300 µl, add the sample to a sterile 15 ml centrifuge tube). ★For frozen blood samples, proceed directly to Step 1 Lysis. For fresh blood samples, add RBC Lysis Buffer which is triple of the sample volume and mix by inversion. **Do not vortex**. And proceed below steps. ★Incubate the tube for 10 minutes at room temperature. ★Centrifuge at 3,000 xg for 5 minutes and **remove the supernatant completely**. ★Add 100 µl of RBC Lysis Buffer to resuspend the cell pellet.

Step 1 **Lysis**	**Tissue** ★Add **500 µl of TR1 Buffer** and 8 µl of ß-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved. ★Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
	Cultured Animal/Bacterial Cells ★Add **500 µl of TR1 Buffer** and 8 µl of ß-mercaptoethanol to the sample and mix completely.
	Fresh Blood/Frozen Blood ★Add **500 µl of TR1 Buffer** and 8 µl of ß-mercaptoethanol to the fresh blood/frozen blood sample and mix completely.
	Serum ★Add **500 µl of TR1 Buffer** and 8 µl of ß-mercaptoethanol to 100 µl of serum and mix completely.
	★Incubate at 70ºC for 10 minutes (Only for frozen blood samples, incubate at 90ºC for 30 minutes). ★Incubate at 15-30ºC for 5 minutes. ★Microcentrifuge at 13,000rpm at 2-8ºC for 15 minutes. ★Transfer the supernatant to a new 1.5 ml microcentrifuge tube and add **TR2 Buffer which is 1/10 volume of the supernatant.**
Step 2 **Isolation**	★Add 500 µl of chloroform to the mixture from Step 1. ★Shake vigorously and then centrifuge at full speed for 5 minutes. ★Carefully remove the upper layer and transfer it to a new 1.5 ml microcentrifuge tube (repeat the Isolation Step until the interphase becomes clear).
Step 3 **RNA** **Precipitation**	★Carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube containing 500 µl of Isopropanol. ★Gently invert the tube 3-5 times. ★Incubate on ice for 10 minutes. ★Centrifuge at full speed for 15 minutes. ★Discard the supernatant and wash the pellet with 1 ml of 70% ethanol. ★Centrifuge at 2-8ºC at full speed for 5 minutes. ★**Completely discard the supernatant** and add 50-100 µl of RNase-free water to the 1.5 ml microcentrifuge tube. ★Incubate for 10 minutes at 60ºC to dissolve the pellet.

Yeast Protocol

Additional Requirements:

RNase-free microcentrifuge tubes, absolute ethanol for preparing 70% ethanol in RNase-free water, Chloroform, Isopropanol, mortar and pestle, ß-mercaptoethanol, RNase-free water, sorbitol buffer (1.2 M sorbitol; 10 mM CaCl2; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol). Solutions (water and other solutions) should be treated with 0.1% DEPC.

Notes:

If a larger sample volume is required, scale the TR1 Buffer proportionately. For complete DNA Degradation, add 2µl 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 25ºC} to the final sample in the RNA Precipitation Step. Let stand for 10 minutes at room temperature.

Sample Preparation Steps for Yeast Sample

1. Harvest fungus cells (up to 5 x 10^7) by centrifugation for 10 minutes at 5,000 x g.
2. Discard the supernatant and resuspend the pellet in 600 µl of sorbitol buffer.
3. Add 200 U of lyticase or zymolase. Incubate at 30ºC for 30 minutes.
4. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast.
5. Remove the supernatant and add 20 µl of RNase-free water to resuspend the pellet.
6. Then follow the lysis step of Cultured Animal/Bacterial Cells protocol. Add 500 µl of TR1 Buffer and 8 µl of ß-mercaptoethanol to the sample and mix completely.