HiYield™ Total RNA Maxi Kit (Tissue)

Cat. No.:	YRTM10	YRTM25
Product Name:	HiYield™ Total RNA Maxi Kit (Tissue)	
Reactions:	10	25
Sample:	Up to 200 mg of tissue or paraffin-embedded tissue	
Yield:	Up to 300 ug	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 60 Minutes	

Introduction

HiYield™ Total RNA Maxi Kit (Tissue) is designed specifically for purifying total RNA from a variety of animal tissue and paraffin-embedded tissue. Provided micropestles can efficiently homogenize tissue samples to shorten the time in the Lysis Step. The entire procedure can be completed within 1 hour without phenol/chloroform extraction or alcohol precipitation. The purified total RNA is ready for use in RT-PCR, Northern Blotting, Primer Extension and cDNA Library Construction.

Features

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

Components

ITEM	YRTM10	YRTM25
RB Buffer	60ml	130ml
W1 Buffer	50ml	130ml
Wash Buffer*	25ml	62.5ml
RNase-Free Water	6ml	30ml
RTM Column	10pcs	25pcs
Lysate Filter 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 (Tissue) is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 100 mg of mouse liver. More than 100 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.

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}

Optional Steps of DNA Residue Degradation:

Optional Step 1:

Add 100 µl of 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} to the center of the RTM Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

Optional Step 2:

Add 2 µl of 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} to the final elution sample. Let stand for 10 minutes at room temperature.

Step 1 Tissue Dissociation	★Cut off up to 200 mg of fresh or frozen animal tissue. (If using frozen animal tissue, the sample MUST have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use in order to avoid RNA Degradation). ★Grind the sample (in liquid nitrogen) to a fine powder. Transfer the powder to a 15 ml centrifuge tube.
Step 2 Lysis	★Add **5 ml of RB Buffer** and 50 µl of ß-mercaptoethanol to the ground sample and mix by vortex. Incubate at room temperature for 5 minutes. ★Place a **Lysate Filter Column** in a 50 ml centrifuge tube and transfer the sample lysate to the column. Centrifuge for 5 minutes at full speed. ★Discard the **Lysate Filter Column** and Proceed to Step 3 RNA Binding.
Step 3 RNA Binding	★Add a half volume of absolute ethanol to the clarified filtrate from Step 2 and vortex immediately (ex: add 2.5 ml of absolute ethanol to 5 ml of filtrate). ★Place a **RTM Column** in a 50 ml centrifuge tube. ★Transfer the ethanol-added mixture to the **RTM Column**. ★Centrifuge at full speed for 5 minutes and discard the flow-through **(if the mixture could not flow through the RTM Column membrane following centrifugation, increase the centrifuge time until the mixture passes completely)**.
	Optional Step 1 of DNA Residue Degradation **(see optional steps of DNA Residue Degradation in page 2)**
Step 4 Wash	★Add **4 ml of W1 Buffer** to the center of the **RTM Column**. ★Centrifuge at full speed for 3 minutes. Discard the flow-through and place the **RTM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) to the center of the **RTM Column**. Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RTM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) to the center of the **RTM Column**. Centrifuge at full speed for 3 minutes. ★Discard the flow-through and place the **RTM Column** back in the 50 ml centrifuge tube. Centrifuge at full speed for 10 minutes to dry the column matrix.

Step 5 RNA Elution	★Place the dried **RTM 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.
	Optional Step 2 of DNA Residue Degradation **(see optional steps of DNA Residue Degradation in page 2)**

Troubleshooting

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