HiYield Total RNA Mini Kit (Tissue)

Cat. No.:	YRT50	YRT100	YRT300
Product Name:	HiYield Total RNA Mini Kit (Tissue)		
Reactions:	50	100	300
Sample:	Up to 25 mg of Tissue or Paraffin-Embedded Tissue		
Yield:	Up to 100 ug		
Format:	Spin Column		
Operation:	Centrifuge		
Operation Time:	Within 60 Minutes		

Introduction

HiYield Total RNA Mini 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.

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.

Components

ITEM	YRT50	YRT100	YRT300
RB Buffer	30ml	60ml	130ml
W1 Buffer	30ml	50ml	130ml
Wash Buffer*	12.5ml	25ml	100ml
RNase-Free Water	6ml	15ml	30ml
RT Column	50pcs	100pcs	300pcs
Lysate Filter Column	50pcs	100pcs	300pcs
2 ml Collection Tube	100 pcs	200 pcs	600pcs
Micropestle	50pcs	100pcs	300pcs

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

Features

Ready-to-use RNA for many downstream applications.

Excellent Recovery of RNA.

Complete removal of contaminants and inhibitors.

Quality Control

The quality of HiYield™ Total RNA Mini Kit (Tissue) is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 10 mg mouse liver. More than 20 µg of total RNA was quantified with a spectrophotometer and checked by formaldehyde agarose gel analysis. 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.

Tissue Protocol

Things to do before starting

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

Additional requirements: 20-G needle syringe, absolute ethanol, microcentrifuge tubes (RNase free), ß-mercaptoethanol.

Optional additional requirements: 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 Lysis**	★Cut off up to 25 mg of fresh or frozen animal tissue and transfer it to a 1.5 ml microcentrifuge tube (If using frozen animal tissue, the sample MUST have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use, to avoid RNA Degradation). ★Add **400 µl** of **RB Buffer** and 4 µl of ß-mercaptoethanol to the tube and use the provided **Micropestle** to grind the tissue a few times. ★Shear the tissue by passing lysate through a 20-G needle syringe 10 times. ★Incubate at room temperature for 5 minutes. ★Place a **Lysate Filter Column** in a **2 ml Collection Tube** and transfer the sample mixture to the **Lysate Filter Column**. ★Centrifuge for 1 minute at 1,000 x g. ★Discard the **Lysate Filter Column** and proceed to Step 2 RNA Binding.
Step 2 **RNA** **Binding**	★Add 400 ul of 70% absolute ethanol to the filtrate from Step 1 and shake vigorously (break up any precipitate with pipetting). ★Place a **RT Column** in a **2 ml Collection Tube**. ★Transfer the ethanol-added mixture to the **RT Column**. ★Centrifuge at full speed for 2 minutes (if the lysate mixture could not flow past the **RT Column** membrane following centrifugation, increase the centrifuge time until the lysate mixture passes completely). ★Discard the flow-through and place the **RT 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 **RT Column** matrix. ★Let stand for 10 minutes at room temperature.

Step 3 **Wash**	★Add **400 µl of W1 Buffer** to the **RT Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **RT Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **RT Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RT Column** back in the **2 ml Collection Tube**. ★Centrifuge again for 3 minutes at full speed to dry the column matrix.
Step 4 **RNA Elution**	★Place the dried **RT 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.

Paraffin-Embedded Tissue Protocol

Things to do before starting

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

Additional requirements: xylene, microcentrifuge tubes, absolute ethanol.

Optional additional requirements: 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 Tissue Dissociation	★Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube. ★Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation. ★Centrifuge at full speed for 3 minutes. Remove the supernatant. ★Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting. ★Centrifuge at full speed for 3 minutes. Remove the supernatant. ★Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting. ★Centrifuge at full speed for 3 minutes. Remove the supernatant. ★Open the tube and Incubate at 37ºC for 15 minutes to evaporate any ethanol residue.
Step 2 Cell Lysis	★Cut off up to 25 mg of fresh or frozen animal tissue and transfer it to a 1.5 ml microcentrifuge tube (If using frozen animal tissue, the sample MUST have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use, to avoid RNA Degradation). ★Add **400 µl** of **RB Buffer** and 4 µl of ß-mercaptoethanol to the tube and use the provided **Micropestle** to grind the tissue a few times. ★Shear the tissue by passing lysate through a 20-G needle syringe 10 times. ★Incubate at room temperature for 5 minutes. ★Place a **Lysate Filter Column** in a **2 ml Collection Tube** and transfer the sample mixture to the **Lysate Filter Column**. ★Centrifuge for 1 minute at 1,000 x g. ★Discard the **Lysate Filter Column** and proceed to Step 2 RNA Binding.

Step 3 **RNA** **Binding**	★Add 400 ul of 70% absolute ethanol to the filtrate from Step 1 and shake vigorously (break up any precipitate with pipetting). ★Place a **RT Column** in a **2 ml Collection Tube**. ★Transfer the ethanol-added mixture to the **RT Column**. ★Centrifuge at full speed for 2 minutes (if the lysate mixture could not flow past the **RT Column** membrane following centrifugation, increase the centrifuge time until the lysate mixture passes completely). ★Discard the flow-through and place the **RT 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 **RT Column** matrix. ★Let stand for 10 minutes at room temperature.
Step 4 **Wash**	★Add **400 µl of W1 Buffer** to the **RT Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **RT Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RT Column** back in the **2 ml Collection Tube**. ★Add **600 µl of Wash Buffer** (ethanol added) to the **RT Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **RT Column** back in the **2 ml Collection Tube**. ★Centrifuge again for 3 minutes at full speed to dry the column matrix.
Step 5 **RNA Elution**	★Place the dried **RT 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 RT 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 RT Column membrane ★Ethanol carryover
RNA Degradation	★Harvested sample not immediately stabilized ★Inappropriate handling of starting material ★RNase contamination