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TRIeasy™ Total RNA UltraPurification Kit (Blood/Bacteria/Cultured Cells/Tissue)

Cat. No.:	TTU050	TTU100	TTU200
Product Name:	TRIeasy™ Total RNA UltraPurification Kit (Blood/Bacteria/Cultured Cells/Tissue)		
Product Size:	50 preps	100 preps	200 preps
Sample Type:	Up to 200µl of blood, buffy coat, plasma, serum, up to 5 x 10^6 of cells, 10-50 mg of tissue, up to 1 x 10^9 bacteria cells, or 20-50 mg of plant tissue.		
Final Product:	Total RNA		
Format:	Organic homogenization + spin column purification		
Operation:	Centrifuge		
Operation Time:	Within 15 minutes		

Introduction

TRIeasy™ Total RNA UltraPurification Kit (Blood/Bacteria/Cultured Cells/Tissue) is designed for the isolation of total RNA from samples of human, animal, plant, yeast or bacterial origin. Sample is first homogenized with TRIeasy™ Reagent (without chloroform phase separation or isopropanol RNA precipitation). Then the sample is transferred to spin column containing a clear silica-based membrane to which the RNA binds during purification. The RNA is then completely washed to remove contaminants and the purified total RNA is finally eluted in RNase-Free Water.

Without chloroform phase separation or isopropanol RNA precipitation, spin column purification largely shorten the purification time and increase the purity. Furthermore, there will be no phenol carryover!

Purification time is merely 15 minutes. The ultra-pure RNA can be utilized directly in a wide range of sensitive downstream applications, such as gene expression studies, microarray analysis or real time quantitative RT-PCR. TRIeasy™ Total RNA UltraPurification Kit (Blood/Bacteria/Cultured Cells/Tissue) provides a simple, rapid and reliable method which fulfills the need for high quality nucleic acid, reproducible purification, ease of use and increased throughput for research laboratories.

Features

- Purification of high yield, ultra-pure total RNA within 15 minutes.
- No chloroform phase separation or isopropanol RNA precipitation.
- No phenol carryover.
- Ultra-pure RNA with A260/A280 >1.8, A260/A230 >1.8.

Applications

Ultra-pure RNA is ready for direct use in cDNA library construction, qRT-PCR, nuclease protection assays, RNA amplification for microarray analysis, northern blotting, dot blot hybridization, in vitro translation or poly(A)+ selection.

Quality Control

TRIeasy™ Total RNA UltraPurification Kit (Blood/Bacteria/Cultured Cells/Tissue) is tested on a lot-to-lot basis. 10 µl from a 50 µl eluate of purified RNA is analyzed by electrophoresis on a 0.8% agarose gel.

Shipping and Storage Conditions

TRIeasy™ Total RNA UltraPurification Kit (Blood/Bacteria/Cultured Cells/Tissue) is shipped at room temperature and should be stored dry at 2ºC to 25ºC. With proper storage, TRIeasy™ Total RNA UltraPurification Kit (Blood/Bacteria/Cultured Cells/Tissue) can be stored for up to 9 months without showing any deduction in performance and quality.

Product Components and Storage Conditions

Cat. No.:	TTU050	TTU100	TTU200	Storage Conditions
TRIeasy™ Reagent	40 ml	80 ml	160 ml	Store dry at 2ºC to 25ºC
W1 Buffer*	21 ml	35 ml	70 ml	Store dry at room temperature (15ºC-25ºC)
Wash Buffer (concentrated)*	25 ml	50 ml	75 ml	Store dry at room temperature (15ºC-25ºC)
RNase-Free Water	6 ml	6 ml	15 ml	Store dry at room temperature (15ºC-25ºC)
RB Column:	50 pcs	100 pcs	200pcs	Store dry at room temperature (15ºC-25ºC)
2 ml Collection Tube	100 pcs	200 pcs	400pcs	Store dry at room temperature (15ºC-25ºC)

*Add absolute ethanol (see the bottle label for volume) to W1 Buffer and Wash Buffer, and then mix by shaking for a few seconds. Check the box on the bottles. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Caution

TRIeasy™ Reagent contains phenol and guanidine isothiocyanate. During operation, always work in a fume hood, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask. Disposable/non-disposable glassware, plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.

If contact to skin or eyes occurs, immediately wash the exposed area with copious amounts of water for 15 minutes and seek medical attention if necessary. If you inhale vapors, move to fresh air and seek medical attention if necessary.

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Protocol: RNA Purification

Please read the entire instruction manual prior to starting the Protocol Procedure.

Materials to be supplied by user:

- Absolute ethanol, lysozyme and bacteria lysis buffer (bacteria only), 1.5 ml microcentrifuge tubes(RNase-free)
- Optional：DNase I

Step 1 Sample Homogenization and Lysis	Sample preparation should be performed at room temperature. Please follow the steps below for specific sample preparation. To avoid DNA contamination of extracted RNA, be sure and use the indicated volume of TRIeasy™ Reagent. Lysozyme (YLY500, 500mg) and Bacteria Lysis Buffer (YRC-BLB) can be purchased directly from Real Biotech Corp.
	Adherent Cultured Cells 1. Remove the culture medium from culture dish. 2. Directly add 100 µl of TRIeasy™ Reagent per cm2 of culture dish surface area. 3. Lyse the cells directly in the culture dish by pipetting several times. 4. Incubate the sample mixture for 5 minutes at room temperature. 5. Transfer the sample to a 1.5 ml microcentrifuge tube (RNase-free).
	Suspension Cultured Cells (up to 5 x 10^6) 1. Transfer cells (up to 5 x 10^6) to a 1.5 ml microcentrifuge tube (RNase-free). 2. Harvest by centrifugation at 300 x g for 5 minutes then remove the culture medium completely. 3. 700 µl of TRIeasy™ Reagent should be added to the cell pellet then mixed several times by pipette. 4. Incubate the sample mixture for 5 minutes at room temperature.
	Tissue (10-50 mg) 1. Excise 10-50 mg of tissue directly from the animal or remove the tissue sample from storage. Do not use more than 50 mg of tissue per reaction. 2. Homogenize tissue samples using one of the following methods: A. Transfer the tissue and 700 µl of TRIeasy™ Reagent to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads then homogenize the sample with a TissueLyser, Disruptor Genie or similar. B. Transfer the tissue and 700 µl of TRIeasy™ Reagent to a 1.5 ml centrifuge tube and grind the tissue with a micropestle a few times then shear the tissue by passing the lysate through a 20-G needle syringe 10 times. C. Transfer the tissue and 700 µl of TRIeasy™ Reagent to a glass-Teflon or Polytron

	homogenizer. Transfer the homogenized sample to a 1.5 ml microcentrifuge tube (RNase-free). 3. Incubate the homogenized sample for 5 minutes at room temperature.
Step 1 Sample Homogenization and Lysis	**Body Fluids (blood, buffy coat, plasma, serum) (up to 200 µl)** 1. Transfer up to 200 µl of liquid sample to a 1.5 ml of microcentrifuge tube (RNase-free). 2. Add 3 volumes of TRIeasy™ Reagent per 1 volume of sample (TRIeasy™ Reagent : sample= 3:1) then mix well by vortex. 3. Incubate the sample mixture for 5 minutes at room temperature.
	Bacteria (up to 1 x 10^9) 1. Transfer bacteria cells (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube (RNase-free). 2. Centrifuge at 12,000-16,000 x g for 2 minutes then remove the supernatant completely. 3. Weigh and transfer 20 mg of lysozyme powder to a new 1.5 ml microcentrifuge tube (RNase-free). 4. Add 1 ml of bacteria lysis buffer to the microcentrifuge tube containing 20 mg of lysozyme. 5. Vortex the tube until the lysozyme powder is completely dissolved. 6. Add 100 µl of bacteria lysis buffer containing lysozyme to the bacteria cell pellet. 7. Resuspend the cell pellet by vortex or pipetting. NOTE: Residual bacteria lysis buffer containing lysozyme should be stored at 4℃ for 2 weeks. 8. Incubate the sample for 5 minutes at room temperature. 9. Add 700 µl of TRIeasy™ Reagent and mix well by pipette then incubate at room temperature for 5 minutes.
	Plant (20-50 mg) 1. Cut off 20-50 mg of fresh or frozen plant tissue. Do not use more than 50 mg of plant tissue per reaction. 2. Homogenize plant tissue samples using one of the following methods: A. Transfer the plant tissue and 700 µl TRIeasy™ Reagent to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads then homogenize the sample with a TissueLyser, Disruptor Genie or similar. B. Add liquid nitrogen to a mortar (RNase-free) and grind the plant tissue thoroughly using a pestle (RNase-free). Transfer the plant tissue powder and 700 µl of TRIeasy™ Reagent to a 1.5 ml centrifuge tube then vortex briefly. 3. Incubate the homogenized sample for 5 minutes at room temperature.

Step 2 **RNA Binding**	1. Centrifuge the sample at 12,000 -16,000 x g for 1 minute then transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (RNase-free). 2. Add 1 volume of absolute ethanol directly to 1 volume of sample mixture (1:1) in TRIeasy™ Reagent. 3. Mix well by vortex then place a RB Column in a 2 ml Collection Tube. 4. Transfer 700 µl of the sample mixture to the RB Column and centrifuge at 14,000-16,000 x g for 1 minute then discard the flow-through. 5. Repeat the RNA Binding Step by transferring the remaining sample mixture to the RB Column. 6. Centrifuge at 14,000-16,000 x g for 1 minute then discard the flow-through and place the RB Column in a new 2 ml Collection Tube.
(Optional) **Column DNase I** **Digestion Step**	1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14,000-16,000 x g for 30 seconds. 2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube. 3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows: 10X Reaction Buffer : 8 µl DNase I : 3 µl (2 U/µl) RNase free-Water : 69 µl Total Volume : 80 µl 4. Mix the DNase I solution by gentle inversion (DO NOT vortex). 5. Add the DNase I solution (80 µl) into the CENTER of the RB column matrix. 6. Incubate the column for 15 minutes at room temperature (20-30ºC) then centrifuge at 14,000-16,000 x g for 30 seconds. 7. Discard the flow-through then place the RB Column back in the 2 ml collection tube and proceed with RNA Wash.
Step 3 **RNA Wash**	1. Add 400 µl of W1 Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14,000-16,000 x g for 30 seconds. 2. Discard the flow-through then place the RB Column back in the 2 ml Collection Tube. 3. Add 600 µl of Wash Buffer (make sure ethanol was added) to the RB Column. 4. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through and place the RB Column back in the 2 ml Collection Tube. 5. Add 600 µl of Wash Buffer (make sure ethanol was added) to the RB Column. 6. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. 7. Place the RB Column back in the 2 ml Collection Tube. NOTE: (For blood samples only) wash the RB Column again with 600 µl of Wash Buffer. 8. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

Step 4 **RNA Elution**	1. Place the dried RB Column in a clean 1.5 ml microcentrifuge tube (RNase-free). 2. Add 25-50 µl of RNase-free Water into the CENTER of the column matrix. 3. Let stand for at least 3 minutes to ensure the RNase-free Water is absorbed completely by the matrix. 4. Centrifuge at 14,000-16,000 x g for 1 minute to elute the purified RNA.

Troubleshooting

Problem	Cause	Solution
Low Yield	A. Sample lysis or homogenization was incomplete. B. Incorrect RNA elution. C. Precipitates may form during the RNA binding step after adding 1 volume of absolute ethanol to the sample mixture in TRIeasy™ Reagent if too much sample material is used.	A. Starting material should be reduced and completely dissolved in TRIeasy™ Reagent. B. Make sure RNase-free Water is added to the center of the RB Column and is absorbed completely. C. Reduce the sample amount to half of the original.
Degraded RNA	A. Incorrect sample preparation and/or storage. B. Incorrect storage temperature.	A. Process or freeze samples immediately after collection. B. Extracted RNA should be stored at -70ºC.
Low RNA A260/A280	A. Volume of TRIeasy™ Reagent was insufficient for proper sample homogenization. B. Incomplete wash step.	A. Volume of TRIeasy™ Reagent is sample dependent and should be added according to the sample homogenization specifications. B. Wash the RB Column with ethanol added Wash Buffer 3 times.
Eluted RNA does not perform well in downstream applications	Residual ethanol contamination.	Following the wash step, dry the RB Column with additional centrifugation at 14,000-16,000 x g for 5 minutes or incubate at 60℃ for 5 minutes.
Samples were stored in 1 ml of tri-reagent in a 1.5 ml microcentrifuge tube	1 ml (1 volume) of absolute ethanol cannot be added to the same 1.5ml microcentrifuge tube due to the volume limitation of 1.5 ml microcentrifuge tube.	Following centrifugation, transfer the supernatant to a 2 ml or 15 ml centrifuge tube (RNase-free) and add 1 volume of absolute ethanol then mix well by vortex. Transfer 700 µl of the sample mixture to the RB Column then centrifuge and discard the flow-through. Repeat the RNA Binding step until all of the sample mixture has been passed through the RB Column.