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TRIeasy™ Total RNA Isolation Kit (Bacteria)

Cat. No.:	TTB050	TTB100	TTB200
Product Name:	TRIeasy™ Total RNA Isolation Kit (Bacteria)		
Product Size:	50 preps	100 preps	200 preps
Sample Type:	Bacterial cells		
Yield:	High yield and high quality RNA with A260/A280 = 1.8-2.0		
Format:	Organic homogenization + spin column purification		
Operation:	Centrifuge		
Operation Time:	Within 20 minutes		
Elution Volume:	20-50 µl		

Introduction

TRIeasy™ Total RNA Isolation Kit (Bacteria) is designed for the rapid isolation of total RNA from gram-positive and gram-negative bacteria. 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 20 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 Isolation Kit (Bacteria) 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 20 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

The quality of the TRIeasy™ Total RNA Isolation Kit (Bacteria) is tested on a lot-to-lot basis by isolation of RNA from Escherichia coli (1 × 109) culture (OD600=1.3, 1 ml). 10 µl from a 50 µl eluate of RNA is analyzed by electrophoresis on a 0.8% agarose gel.

Shipping and Storage Conditions

TRIeasy™ Total RNA Isolation Kit (Bacteria) is shipped at room temperature and different components should be stored at different temperature. With proper storage, TRIeasy™ Reagent can be stored at 2ºC to 25ºC for up to 12 months without showing any deduction in performance and quality. Bacteria Lysis Buffer can be stored at room temperature (15-25ºC) for up to 12 months, and Lysozyme can be stored at -20ºC for up to 12 months.

Product Components and Storage Conditions

Cat. No.:	TTR050	TTR100	TTR200	Storage Conditions
TRIeasy™ Reagent	40 ml	80 ml	160 ml	Store dry at 2ºC to 25ºC
Bacteria Lysis Buffer	15 ml	15 ml	30 ml	Store dry at room temperature
Lysozyme*	55 mg	110 mg	250 mg	Store dry at -20ºC
W1 Buffer**	21 ml	35 ml	70 ml	Store dry at room temperature
Wash Buffer**	25 ml	50 ml	75 ml	Store dry at room temperature
RNase-Free Water	6 ml	6 ml	15 ml	Store dry at room temperature
RB Column	50 pcs	100 pcs	200 pcs	Store dry at room temperature
2ml Collection Tube	100 pcs	200 pcs	400 pcs	Store dry at room temperature

* Lysozyme is shipped at room temperature and should be stored at -20℃ once received.

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

Caution

TRIeasy™ Total RNA Isolation Kit (Bacteria) 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.*

Protocol: RNA Extraction

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

Materials to be supplied by user:

- Absolute ethanol, 1.5 ml microcentrifuge tubes (RNase-free).
- Optional：DNase I, 1 µL of 20 mM EGTA (pH=8.0) for Optional Step 2: DNA Digestion in Solution.

Step 1 Sample Preparation and Lysis	Sample preparation should be performed at room temperature. To avoid DNA contamination of extracted RNA, be sure and use the indicated volume of TRIeasy™ Reagent. 1. Transfer bacterial cells (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube (RNase-free). 2. Centrifuge at 12,000-16,000 xg for 2 minutes then remove the supernatant completely. 3. Weigh and transfer Lysozyme powder (1 mg/sample) to a new 1.5 ml microcentrifuge tube (RNase-free). 4. Add Bacteria Lysis Buffer (100 µl/sample) to the microcentrifuge tube containing 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. 8. Incubate the sample for 5 minutes at room temperature. 9. Add 700 µl of TRIeasy™ Reagent to the sample and mix well by pipetting then incubate at room temperature for 5 minutes.
Step 2 RNA Binding	1. Add 700 µl of absolute ethanol directly to the sample mixture. 2. Mix well by vortex then place a RB Column in a 2 ml Collection Tube. 3. 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. 4. Repeat the RNA Binding Step by transferring the remaining sample mixture to the RB Column. 5. Centrifuge at 14,000-16,000 x g for 1 minute then discard the flow-through. 6. Place the RB Column in a new 2 ml Collection Tube.

(Optional) Column DNase I Digestion Step	DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield. 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: DNase I (2 U/µl) : 5 µl DNase I Reaction Buffer : 45 µl Total Volume : 50 µl 4. Gently pipette the DNase I solution to mix (DO NOT vortex). 5. Add the DNase I solution (50 µl) into the CENTER of the RB column matrix. 6. Incubate the column for 15 minutes at room temperature (20-30ºC) then 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. 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.

(Optional) DNA Digestion In Solution	1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows: RNA in RNase-free water: 1-40 µl DNase I : 0.5 µl/µg RNA DNase I Reaction Buffer : 5 µl RNase-free water: add to final volume = 50 µl Total Volume : 50 µl 2. Gently pipette the DNase I reaction solution to mix (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes. 3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) then incubate the microcentrifuge tube at 65℃ for 10 minutes. NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the YUR100/YUR300 HiYield™ RNA UltraPurification Kit instead of stopping the reaction with EGTA.

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.