www.real-biotech.com
Real Biotech Corporation
13F.-2, No.33, Sec. 1, Minsheng Rd., Banqiao City, Taipei County 220, Taiwan, R. O. C.
Tel: +886 2 2950 9000 Fax: +886 2 2950 0505

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

Cat. No.:	TTR050	TTR100	TTR200
Product Name:	TRIeasy™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue)		
Product Size:	50 preps	100 preps	200 preps
Sample Type:	Blood, buffy coat, plasma, serum, cells and tissue of human, animal, plant, yeast, or bacteria.		
Yield:	High yield and high quality RNA with A260/A280 = 1.7-1.9		
Final Product:	Total RNA/ DNA / Protein		
Format:	Organic Extraction / Reagent (scalable for a wide range of sample sizes)		
Operation:	Centrifuge		
Operation Time:	Within 60 minutes		
Elution Volume:	20-50 µl		

Introduction

TRIeasy™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue) is designed for the isolation of total RNA or the simultaneous isolation of RNA, DNA and proteins from samples of human, animal, plant, yeast or bacterial origin. The entire process is an improvement to the single-step RNA isolation method developed by Chomcynski and Sacchi. After homogenization of sample and chloroform extraction, three phases are formed (aqueous phase, interphase and organic phase). RNA can be precipitated by isopropanol from aqueous phase, DNA can be recovered by ethanol precipitation from interphase, and proteins are precipitated with isopropanol from organic phase.

The extracted RNA can be used directly in a variety of downstream applications such as cDNA Library Construction, Cloning, RT-PCR (Endpoint), Real-Time PCR, Nuclease Protection Assays and Northern Blotting. TRIeasy™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue) is a cost effective alternative for efficient total RNA extraction.

This kit also performs well in sequential precipitation of DNA, and proteins from a single sample. This scalable and simple-to-use format fulfills the need for high quality nucleic acid, reproducible purification, ease of use and increased throughput for research laboratories.

Features

Ready-to use RNA from samples of human, animal, plant, yeast or bacteria within 1 hour.

Sequential precipitation of RNA, DNA and proteins from a single sample.

Easily scalable DNA, RNA and protein isolation.

Reproducible recoveries for constant results.

Applications

Extracted RNA is ready for use in cDNA library construction, qRT-PCR, nuclease protection assays, cloning, RNA amplification for microarray analysis, northern blotting, dot blot hybridization or in vitro translation.

Extracted DNA is ready for use in PCR, southern blots and restriction enzyme digestion.

Extracted protein is ready for use in western blots, recovery of some enzymatic activity and immunoprecipitation.

Quality Control

TRIeasy™ Total RNA Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue) is tested on a lot-to-lot basis by isolation of RNA from 300 µl of human blood samples. 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 (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 Isolation Kit (Blood/Bacteria/Cultured Cells/Tissue) can be stored for up to 12 months without showing any deduction in performance and quality.

Product Components and Storage Conditions

Cat. No.:	TTR050	TTR100	TTR200	Storage Conditions
TRIeasy™ Reagent	50 ml	100 ml	200 ml	Store dry at 2ºC to 25ºC

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.*

Additional Requirements

RNA Extraction: chloroform, isopropanol, 70% ethanol, 1.5 ml microcentrifuge tubes (RNase-free), RNase-free Water.

DNA Extraction: chloroform, absolute ethanol, 70% ethanol, sodium citrate/ethanol solution (0.1 M sodium citrate in 10% ethanol, pH8.5), 8 mM NaOH solution or TE Buffer pH8.5, 1.5 ml microcentrifuge tubes.

Protein Extraction: chloroform, absolute ethanol, 95% ethanol, wash solution (0.3 M guanidine hydrochloride in 95% ethanol), 1% SDS, 15 ml centrifuge tube, 1.5 ml microcentrifuge tubes.

Protocol: RNA Extraction

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

Materials to be supplied by user:

Chloroform, isopropanol, 70% ethanol, RNase-free Water, 1.5 ml microcentrifuge tubes (RNase-free).

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.
	Adherent Cultured Cells 1. Remove the culture medium from culture dish. 2. Directly add 100 µl of TRIeasy™ Reagent per cm^2 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 new 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 and then remove the culture medium completely. 3. Add 1 ml of TRIeasy™ Reagent to the cell pellet and then lyse the cells several times by pipette. 4. Incubate the sample mixture for 5 minutes at room temperature.
	Tissue (50-100 mg) 1. Excise 50-100 mg of tissue directly from the animal or remove the tissue sample from storage. Do not use more than 100 mg of tissue per reaction. 2. Add 1 ml of TRIeasy™ Reagent to 50–100 mg of tissue sample. 3. Homogenize tissue samples using a glass-Teflon or Polytron homogenizer. 4. Incubate the homogenized sample for 5 minutes at room temperature. 5. Transfer the sample to a new 1.5 ml of microcentrifuge tube (RNase-free).
	Body Fluids (blood, buffy coat, plasma, serum) (up to 300 µl) 1. Transfer up to 300 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) and then mix well by vortex. 3. Incubate the sample mixture for 5 minutes at room temperature.

(Optional step)	For samples which contain high levels of fat, proteins, polysaccharides, or extracellular material, perform this optional step following sample homogenization. However, if DNA extraction is required, DO NOT perform this additional step. 1. Centrifuge the sample at 12,000-16,000 x g for 10 minutes to remove insoluble particles. (NOTE: Following centrifugation of high fat content samples, a layer a fat will float on the supernatant. Remove and discard the fatty layer.) 2. Transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (RNase-free). 3. Proceed to Step 2 Phase Separation.
Step 2 Phase Separation	1. Add 200 µl of chloroform to the sample per 1 ml of TRIeasy™ Reagent used in sample homogenization. 2. Shake the microcentrifuge tube vigorously for 10 seconds. 3. Centrifuge the sample at 12,000–16,000 x g for 15 minutes at 4ºC to separate the phases. NOTE: RNA is in the colorless upper aqueous phase which is approximately 50% of the total volume. 4. Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube (RNase-free). NOTE: Be careful not to draw any of the interphase layer (white) or organic phase layer (red) when transferring the aqueous layer. If DNA isolation is required, save the interphase and organic phase then proceed with the DNA Extraction protocol on page 5.
Step 3 RNA Precipitation	1. Add 1 volume of isopropanol to the aqueous phase then mix by inverting the tube several times. 2. Incubate the sample mixture for 10 minutes at room temperature. 3. Centrifuge the sample at 12,000–16,000 x g for 10 minutes at 4ºC to form a tight RNA pellet. 4. Carefully remove and discard the supernatant.
Step 4 RNA Wash	1. Add 1 ml of 70% ethanol to wash the RNA pellet then vortex briefly. 2. Centrifuge the sample at 12,000–16,000 x g for 5 minutes at 4ºC. 3. Being careful not to contact the RNA pellet, remove the supernatant with a pipette. 4. Air-dry the RNA pellet for 5-10 minutes at room temperature. NOTE: **DO NOT** dry the RNA pellet by vacuum centrifuge and avoid over drying the RNA pellet.
Step 5 RNA Resuspension	1. Add 20-50 µl of RNase-free Water to resuspend the RNA pellet. 2. Incubate at 55-60ºC for 10-15 minutes to dissolve the RNA pellet. NOTE: Occasionaly tapping the bottom of the tube during incubation will promote RNA rehydration. The RNA is ready for downstream applications or storage at -70ºC.

Protocol: DNA Extraction

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

Materials to be supplied by user:

Chloroform, absolute ethanol, 70% ethanol, sodium citrate/ethanol solution (0.1 M sodium citrate in 10% ethanol, pH8.5), 8 mM NaOH solution or TE Buffer pH8.5, 1.5 ml microcentrifuge tubes.

NOTE: DNA is extracted from the interphase and organic phase which was saved following the removal of the aqueous phase in the Phase Separation step of RNA Extraction Protocol.

Step 1 **DNA** **Precipitation**	1. Carefully remove any residual upper aqueous phase layer. NOTE: This step is critical for ensuring extracted DNA quality. 2. Add 300 µl of absolute ethanol to the sample per 1 ml of TRIeasy™ Reagent used in sample homogenization. 3. Mix by inverting the tube several times. 4. Incubate the sample for 5 minutes at room temperature. 5. Centrifuge the sample at 2,000 x g for 5 minutes at 4ºC then carefully remove the supernatant. NOTE: If protein isolation is required, save the phenol-ethanol supernatant in a 15 ml centrifuge tube. The supernatant can be stored at -70ºC for several months.
Step 2 **DNA Wash**	1. Add 1 ml of sodium citrate/ethanol solution (0.1 M sodium citrate in 10% ethanol, pH8.5) to the sample per 1 ml of TRIeasy™ Reagent used in initial sample homogenization. 2. Incubate the sample for 30 minutes at room temperature. During incubation, gently invert the tube occasionally. 3. Centrifuge the sample at 2,000 x g for 5 minutes at 4ºC then remove the supernatant. 4. Repeat the above wash steps once. 5. Add 1.5 ml of 70% ethanol to the sample per 1 ml of TRIeasy™ Reagent used in the initial sample homogenization. 6. Incubate for 10-20 minutes at room temperature. During incubation, gently invert the tube occasionally. 7. Centrifuge the sample at 2,000 x g for 5 minutes at 4ºC then carefully remove the supernatant. 8. Air-dry the DNA pellet for 5-10 minutes at room temperature. (**DO NOT** dry the DNA pellet by vacuum centrifuge and avoid over drying the DNA pellet).

Step 3 DNA Resuspension	1. Add 300 µl of 8 mM NaOH solution or TE Buffer pH8.5 to the DNA pellet. NOTE: Resuspending the DNA pellet in a weak base solution is recommended. 2. Incubate the DNA sample at 55-60ºC for 10-15 minutes to dissolve the DNA pellet. NOTE: Occasionaly tapping the bottom of the tube during incubation will promote DNA rehydration. 3. Centrifuge the sample at 12,000-16,000 x g for 10 minutes to remove the insoluble particles. 4. Transfer the supernatant containing the DNA to a new 1.5 ml microcentrifuge tube. The DNA is ready for downstream applications or storage at -20ºC.

Protocol: Protein Extraction

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

Materials to be supplied by user:

Chloroform, absolute ethanol, 95% ethanol, wash solution (0.3 M guanidine hydrochloride in 95% ethanol), 1% SDS, 15 ml centrifuge tube, 1.5 ml microcentrifuge tubes.

NOTE: Protein is extracted from the phenol-ethanol supernatant which was saved from DNA Extraction Protocol.

Step 1 Protein Precipitation	1. Add 1.5 ml of isopropanol to the phenol-ethanol supernatant per 1 ml of TRIeasy™ Reagent used in sample homogenization then mix by inverting the tube several times. 2. Incubate the sample for 10 minutes at room temperature. 3. Centrifuge the sample at 12,000 x g for 10 minutes at 4ºC to precipitate the protein then carefully remove the supernatant.
Step 2 Protein Wash	1. Add 2 ml of wash solution (0.3 M guanidine hydrochloride in 95% ethanol) to the protein pellet per 1 ml of TRIeasy™ Reagent used in the initial sample homogenization. 2. Incubate for 20 minutes at room temperature. The protein sample could be stored in the wash solution for one year at -20ºC. 3. Centrifuge the sample at 7,500 x g for 5 minutes at 4ºC then remove the supernatant. 4. Repeat above wash steps two more times. 5. Add 2 ml of 100% ethanol to the protein pellet after the third wash then vortex. 6. Incubate for 20 minutes at room temperature. 7. Centrifuge the sample at 7,500 x g for 5 minutes at 4ºC then carefully remove the supernatant. 8. Air-dry the protein pellet for 5-10 minutes at room temperature. NOTE: DO NOT dry the protein pellet by vacuum centrifuge and avoid over drying the protein pellet.

Step 3 Protein Resuspension	1. Add 200 µl of 1% SDS to the protein pellet then resuspend the pellet by pipetting. 2. Incubate the sample at 50ºC for 5-10 minutes to completely dissolve the protein pellet. 3. Centrifuge at 10,000 xg for 10 minutes at 4ºC to remove the insoluble particles. 4. Transfer the supernatant containing the protein to a new 1.5 ml microcentrifuge tube. The protein is ready for downstream applications or storage at -20ºC.

Troubleshooting

Problem	Cause	Solution
Low Yield	A. Sample lysis or homogenization was incomplete. B. DNA/RNA/Protein pellet was not dissolved completely.	A. Starting material should be reduced and completely dissolved in TRIeasy™ Reagent. B. Increase incubation temperature to 60ºC and increase incubation time to 15 minutes. If the pellet is still not dissolved, pipette until it dissolves completely.
Degraded DNA/RNA/Protein	A. Incorrect sample preparation and/or storage. B. Incorrect sample storage temperature.	A. Process or freeze samples immediately after collection. B. Extracted RNA should be stored at -70ºC. Extracted DNA and Protein should be stored at -20ºC.
RNA/DNA Contamination	A. When removing the aqueous phase, the interephase and/or organic phase were drawn into the pipette B. The aqueous phase was not removed completely C. The DNA pellet was not washed completely.	A. Leave a small amount of aqueous phase to avoid drawing the interphase and/or organic phase into the pipette. B. Remove the remaining aqueous phase prior to DNA extraction. C. The DNA pellet should be washed with 0.1 M sodium citrate in 10% ethanol pH8.5.
Low RNA A260/A280	A. Volume of TRIeasy™ Reagent was insufficient for proper sample homogenization.	A. Volume of TRIeasy™ Reagent is sample dependent and should be added according to the sample homogenization specifications.
Low DNA A260/A280	A. Residual phenol contamination.	A. The DNA pellet can be washed an additional time with 0.1 M sodium citrate in 10% ethanol pH8.5.