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™ DNA / RNA / Protein Isolation Kit

Cat. No.:	TDR100	TDR200
Product Name:	TRIeasy™ DNA / RNA / Protein Isolation Kit	
Product Size:	100 ml	200 ml
Sample Type:	Cell and tissue samples of human, animal, plant, yeast, or bacterial origin	
Final Product:	DNA, RNA, Protein	
Format:	Reagent	
Operation:	Centrifuge	
Operation Time:	Around 60 Minutes for RNA Isolation	

Introduction

TRIeasy™ DNA / RNA / Protein Isolation Kit 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.

With phenol, guanidine isothiocyanate, unique buffers and stabilizers, this highly reliable kit not only facilitates the immediate and most effective inhibition of RNase activity, but also performs well in sequential precipitation of RNA, DNA, and proteins from a single sample. Furthermore, enhancer buffer is included to eliminate polysaccharides and proteoglycans contamination. The purified RNA can be dissolved easier and it's purer for RT-PCR and northern blot application. The precipitated DNA, or protein are also washed to remove impurities, and then resuspended for use in downstream applications.

Features

- Easily scalable DNA / RNA / Protein isolation.
- Simultaneous isolation of RNA, DNA and proteins from samples of human, animal, plant, yeast or bacteria.
- Better yields than traditional guanidine thiocyanate/cesium chloride methods

Applications

- Isolated RNA can be used in RT-PCR, Northern Blot analysis, Dot Blot hybridization, poly(A)+ selection, in vitro translation, RNase protection assay, and molecular cloning.
- Isolated DNA can be used in PCR, Restriction Enzyme digestion, and Southern Blots.
- Isolated protein can be used for Western Blots, recovery of some enzymatic activity, and immunoprecipitation.

Shipping and Storage Conditions

TRIeasy™ DNA / RNA / Protein Isolation Kit is shipped at room temperature and should be stored immediately upon receipt at 4℃ in a constant temperature freezer. With proper storage, TRIeasy™ DNA / RNA / Protein Isolation Kit can be stored for up to 12 months without showing any deduction in performance and quality.

Product Components

Cat. No.:	TDR100	TDR200
TRIeasy™ DRP Buffer	100 ml	200 ml
Enhancer Buffer	30 ml	60 ml

Caution

TRIeasy™ DNA / RNA / Protein Isolation Kit contain phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Always work with TRIeasy™ DNA / RNA / Protein Isolation Kit in a fume hood. During operation, always wear a lab coat, disposable gloves, and protective goggles. Avoid breathing vapor. Avoid direct contact with TRIeasy™ DNA / RNA / Protein Isolation Kit, because contact to skin, eyes, or respiratory tract may cause chemical burns to the exposed area.

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.

Materials Needed

The following additional materials are needed, but not supplied in this kit.

RNA Isolation	DNA Isolation	Protein Isolation
● Chloroform (chilled) ● Isopropanol (chilled) ● 75% ethanol (in DEPC-treated water or RNase- free water) ● RNAstill™ RNA Storage Solution (Cat. No. YRR001/YRR010) or RNase-free water or 0.5% SDS solution or deionized formamide	● 100% ethanol ● 0.1 M sodium citrate in 10% ethanol ● 75% ethanol ● 8 mM NaOH ● 0.1 M or HEPES (free acid), no pH adjustment ● 0.1 M EDTA (pH 8.0)	● Isopropanol ● 0.3 M guanidine hydrochloride in 95% ethanol ● Ethanol ● 1% SDS

Protocol: RNA Isolation

Materials to be supplied by user:

- Chloroform (chilled).
- Isopropanol (chilled).
- 75% ethanol (in DEPC-treated water or RNase- free water).
- RNAstill™ RNA Storage Solution (Cat. No. YRR001/YRR010) or RNase-free water or 0.5% SDS solution or deionized formamide.

Important Notes:

- Incomplete homogenization will cause contamination of genomic DNA and RNA degradation after isolation.
- For small sample, 1-10 mg of tissues or 10^2-10^4 of cells, use only 800 µl of TRIeasy™ DRP Buffer.
- For some samples such as muscles tissue and tuberous parts of plants which contain high amount of protein, fat, polysaccharides or extracellular materials may not be dissolved in homogenate, it is necessary to spin down this insoluble materials by 12,000 x g for 10 minutes at 4℃. Transfer the supernatant to a new tube for the next step.
- The homogenate can be stored for one month at -70°C before isolation.
- Two major sources of RNase contamination are fingers and dust. Wear gloves at all times and keep samples covered.

Step 1 Homogenization	**Animal and Plant Tissues (10-100 mg)** Homogenize 10-100mg of tissues samples with 1 ml of TRIeasy™ DRP Buffer with a few strokes in a glass-teflon homogenizer or polytron homogenizer for 15-30 seconds until complete lysis. Make sure the complete homogenization is done.
	Suspension Cells (5 x 10^6–1 x 10^7) To isolate RNA from cells grown in suspension, sediment cells and discard the culture medium, lyses them by the addition of 1 ml of TRIeasy™ DRP Buffer to 5x10^6–1x10^7 of animal and plant cells. Pass the cell lysate several times through a pipette until complete lysis.
	Monolayer Cells (per 10 cm^2 dish) To isolate RNA from cells grown in monolayer, remove and discard the culture fluid. Lyse cells by adding directly to the culture dish or flask 1 ml of TRIeasy™ DRP Buffer per 10 cm^2 dish (not according to the cell numbers as suspension cells). Pass the cell lysate several times through a pipette until complete lysis. Be sure the complete homogenization.

	Bacterial Culture (up to 1 x 10^8) ★Before starting: prepare lysozyme solution (4 mg/ml) in TE buffer. a. Transfer bacterial culture (up to 10^8 cells at log phase) to pre-chilled microcentrifuge tube. b. Spin down the cells at 6,000 x g for 5 minutes at 4℃. c. Discard the supernatant and add 100 µl of freshly prepared lysozyme solution to the pellet. Mix well by gently pipetting up and down. Incubate at room temperature for 10-30 minutes. d. Add 1 ml of TRIeasy™ DRP Buffer to the cells and pass the cell lysate several times through a pipette until complete lysis. e. Spin down the insoluble material at 12,000 x g for 10 minutes at 4℃. Transfer the supernatant to a new tube.
Step 2 Phase Separation	★Incubated the homogenate for 5 minutes at room temperature to completely dissociate the nucleoprotein complex. ★Add 0.2 ml of chloroform per 1 ml of TRIeasy™ DRP Buffer. Cap and shake vigorously for 15 seconds and incubate them at room temperature for 2-3 minutes. ★Centrifuge the samples at 12,000 x g for 15 minutes at 4℃. After centrifugation, the mixture separates into a yellow phenol chloroform phase, an interphase and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase (with a volume of approximately 0.6 ml per 1 ml of TRIeasy™ DRP Buffer), DNA is in interphase and proteins are in organic phase. ★Carefully transfer the aqueous phase to a new microcentrifuge tube without disturbing or touching the interphase. If interphase is disturbing by pipetting or contamination of interphase needs to be minimum, spin the transferred aqueous phase at 12,000 x g for 10 minutes at 4℃, transfer the supernatant to a new tube.

Step 3 RNA Precipitation	★Important info before starting: a. Enhancer Buffer will greatly reduce contamination of polysaccharides and proteoglycans which co-precipitate with RNA. Some small RNA < 200 nt RNA (i.e., tRNA, 4S RNA) will also be removed. If these RNA are interested, skip the addition of Enhancer Buffer and adding 0.5 ml of isopropanol to precipitate RNA in stead. b. When precipitating RNA from small sample quantities (<10^4 cells or <10 mg tissue), add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase before isopropanol precipitation. c. If DNA or Protein isolation is necessary, store the remaining interphase and organic phase solution at -20℃. ★Add 0.25 ml of isopropanol and 0.25 ml of Enhancer Buffer per 1 ml of TRIeasy™ DRP Buffer and mix gently, store samples for 10 minutes at room temperature. ★Centrifuge the samples at 12,000 x g for 10 minutes at 4℃ (RNA precipitate forms a white pellet at the bottom of the tube).
Step 4 RNA Wash	★Carefully remove the supernatant, wash the RNA pellet in 1 ml of 75% ethanol per 1 ml of TRIeasy™ DRP Buffer. Mix the sample by vortexing and centrifuge at 12,000 x g for 5 minutes at 4℃.(RNA sample can be stored in 75% ethanol for one week at 4℃ or one year at -20℃.) ★Remove the ethanol and air dry the RNA pellet briefly for 5-10 minutes. (Do not dry the RNA pellet by SpeedVac, which will make RNA hard to be dissolved.)
Step 5 RNA Resuspension	★Dissolve RNA in 1X RNAstill™ RNA Storage Solution (Cat. No. YRR001, YRR010) or RNase-free water or 0.5% SDS solution or deionized formamide by repetitive pipetting, and incubate for 20 minutes at 65℃. Store the samp les at -20℃ if stored at RNAstill ™ RNA Storage Solution or deionized formamide. Store the sample at -70℃ if store at RNase-free water or 0.5% SDS solution. *RNAstill™ RNA Storage Solution contains mixture of non-toxin chemicals, which will disrupt RNase and protect RNA from degradation even at room temperature or 37℃, and stored RNA can be used at many applications including RT-PCR and northern blot. *RNA store at deionized formamide can also be used in some RT-PCR and northern blot. *RNA store at 0.5% SDS can only be used in northern blot.

Protocol: DNA Isolation

Materials to be supplied by user:

- 100% ethanol.
- 0.1 M sodium citrate in 10% ethanol.
- 75% ethanol.
- 8 mM NaOH.
- 0.1 M or HEPES (free acid), no pH adjustment.
- 0.1 M EDTA (pH 8.0).

Step 1 DNA Precipitation	★For the remaining interphase and organic phase from RNA isolation, remove the aqueous phase completely by carefully pipetting, which reduces the RNA contamination to minimum. Careful removal of any residual aqueous phase is critical for the quality of the isolated DNA. ★Add 0.3 ml of 100% ethanol per 1 ml of TRIeasy™ DRP Buffer to the interphase and organic phase and mix by inverting several times.. ★Incubate the mixture for 2-3 minutes at room temperature, then centrifuge at ≤ 2,000 xg for 5 minutes at 4℃ (centrifugation for too high speed may cause DNA shearing). ★A white DNA pellet will be formed at the bottom of the tube. If protein isolation is desired, transfer the supernatant to a new tube and store at 4℃. Otherwise, discard the supernatant.
Step 2 DNA Wash	★Add 1 ml of 0.1 M Sodium Citrate in 10% ethanol per 1 ml of TRIeasy™ DRP Buffer to the DNA pellet. ★Incubate the solution at room temperature for 30 minutes, vortex several times during incubation to remove the phenol from pellet, then centrifuge at ≤ 2,000 x g for 5 minutes at 4°C. ★Wash one or two more times with 0.1 M Sodium Citrate in 10% ethanol (repeat above 2 steps one or two more times). ★After two or three washes, add 1.5 ml of 75% ethanol and incubate at room temperature for 10 minutes and vortex 2-3 time during incubation, then centrifuge at ≤ 2,000 x g for 5 minutes at 4°C.

Step 3 DNA Suspension	★Remove the supernatant, air dry the pellet for 5-10 minutes. Add 300-600 μl of 8 mM NaOH to dissolve the DNA pellet (DNA will not be dissolved in water or TE buffer), incubate at 60°C and flicking the tube from time to time to facilitate DNA to dissolve. *Do not dry the DNA pellet by SpeedVac, which will make DNA hard to be dissolved. *DNA in 8 mM NaOH can only be stored overnight at 4°C. *DNA may contain insoluble material, remove it by spin at 12,000 x g for 10 minutes and transfer the supernatant to a new tube. ★Adjust the pH of DNA solution to pH 7.5-8.0 by adding 12 μl of 0.1 M HEPES and 1 μl of 0.1 M EDTA per 100 μl of DNA solution. Now the DNA can be stored at -20°C for months and ready for PCR or restriction digestion.

Protocol: Protein Isolation

Materials to be supplied by user:

- Isopropanol.
- 0.3 M guanidine hydrochloride in 95% ethanol.
- ethanol.
- 1% SDS.

Step 1 Protein Precipitation	★For the phenol-ethanol supernatant, add 1.5 ml of isopropanol to the solution per 1 ml of TRIeasy™ DRP Buffer (if using 1.5 ml tube, separate the phenol-ethanol supernatant into two 1.5 ml tubes, and add 0.75 ml of isopropanol to each tube). ★Incubate the mixture for 10 minutes at room temperature, and then spin at 12,000 x g for 10 minutes at 4℃.
Step 2 Protein Wash	★Remove the supernatant and wash the pellet by 2 ml of 0.3 M Guanidine hydrochloride in 95% ethanol per 1 ml of TRIeasy™ DRP Buffer (if using 1.5 ml tube, add 1 ml of 0.3 M guanidine hydrochloride in 95% ethanol to each tube). ★Incubate the solution at room temperature for 20 minutes, vortex several times during incubation to remove the phenol from pellet, then centrifuge at 7,500xg for 5 minutes at 4°C. ★Repeat above 2 wash steps two more times, and discard the supernatant. ★Add 2 ml of ethanol to the pellet (if using 1.5 ml tube, add 1 ml of ethanol to each tube), incubate for 20 minutes at room temp., then centrifuge at 7,500 x g for 5 minutes at 4°C.
Step 3 Protein Suspension	★Air dry the pellet for 5-10 minutes, dissolve the protein pellet in 100-200 μl of 1% SDS at 50°C. Store the protein sample at -20°C and the protein sample is ready for use in PAGE/Western blotting. (Solution may contain insoluble material, remove it by spin at 12,000 x g for 10 minutes and transfer the supernatant to a new tube)