DNAbiotech

Biotechnology is our expertise

TrizoLEX

Catalog no.: DB9683

10, 25, 50 & 100 ml

Related product: DEPC treated water

Intended for Research Use Only

Modares Technology and Sciences Park, Room 510. No. 15, Gordafarid-heyat junction, North Kargar, Enghelan Square, Tehran, I.R. Iran

Www.dnabiotch.ir

Cell phone: +989128382915

Diba NoAvaran Azma Company

Customer and technical support

If you have any question, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

Contact us at
www.dnabiotech.ir
Tell: +989128382915
E-mail: dnabiotechco@gmail.com

Quality Control
In accordance with DNAbiotech Co. Management System, each part of the product tested against predetermined specifications to ensure consistent product quality**.**

Safety Notes

The **TrizoLEX** contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Exposure to **TrizoLEX** can be a serious health hazard. Exposure can lead to serious chemical burns and permanent scarring care should be taken during handling. Always wear gloves and eye protector, a lab coat and a plastic apron are recommendedtoo. It also contains chaotropes agents. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste**.** So**,** follow standard safety precautions.

General description

DNAbiotech **TrizoLEX**, a mono-phasic solution of phenol and guanidine isothiocyanate, is a ready to use reagent for the isolation of total RNA from cells and tissues. This reagent is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi.

As the samples are homogenized or be lysed, **TrizoLEX** maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation. Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropyl alcohol yields proteins from the organic phase.

Copurification of the DNA may be useful for normalizing RNA yields from sample to sample. This technique performs well with small quantities of tissue (50-100 mg) and cells (5×10^6), and large quantities of tissue (≥1 g) and cells ($>10^7$), of human, animal, plant, or bacterial origin. The simplicity of the **TrizoLEX** Reagent method allows simultaneous processing of a large number of samples. The entire procedure can be completed in about one hour. Total RNA isolated by **TrizoLEX** reagent is free of protein and DNA contamination. It can be used for Northern blot analysis, dot blot hybridization, poly (A)+ selection, in vitro translation, RNase protection assay, and molecular cloning. For use in the polymerase chain reaction (PCR), treatment of the isolated RNA with RNase free grade “DNase I” is recommended when the two primers lie within a single exon.

Product Information

Cat #: DB9683 (10, 25, 50 and 100 ml)
Form: Liquid
Featured industry: For Research Use Only
Shipped in: Wet ICE
Min shelf time: 24 months
Storage condition: 4° C

Downstream applications: RT-PCR, cDNA synthesis, Northern, dot, and slot blot analyses, Primer extension, Poly A+ RNA selection etc.

Before Start

Note: To prevent RNase contamination, always wear gloves. Use sterile, disposable plastic ware and pipettes dedicated strictly to RNA work to prevent cross-contamination with RNases from shared equipment.

Sample Types

Sample	Qty.	Volume of TrizoLEX
Whole blood	500µl (up to 2 ml)	1 ml
Adherent cells	10 cm^2 or up to 10^7 cells	1-2 ml
Suspension cells	Up to 10^7 cells	1 ml
Oridinary tissue	Up to 100 mg	1 ml
Special tissue (cartilage, bone, spleen and liver)	50-100 mg	1-2 ml
Bacterial cells	Up to 1×10^7 cells	1 ml
Plant and Fungi	Up to 100 mg	1 ml
Liquid materials (like serum)	100 µl	1 ml

Note: Use fresh blood sample for RNA blood extraction. Prior to RNA extraction it would be recommended that RBCs be removed by RBC Lysis Buffer and white blood cells (WBC pellet) used as starting material.

Reagents (Should be provide before start)

Chloroform (DB9653-100ml)

Isopropanol (DB9680-100 ml)

DEPC Water (DB9616)

75% Ethanol

Important note (HOMOGENIZATION):

Disruption and homogenization of sample are two distinct and essential steps for RNA isolation. Insufficient disruption and homogenization significantly will reduce RNA yield.

Sample Disruption/Homogenization methods:

Adherent Cells: Lyse cells directly in a culture dish by adding 1 ml of **TrizoLEX** Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of **TrizoLEX** Reagent added is based on the area of the culture dish (1 ml per 10 cm^2) and not on the number of cells present. An insufficient amount of **TrizoLEX** Reagent may result in contamination of the isolated RNA with DNA.

Suspension Cells: Pellet cells by centrifugation. Lyse cells in **TrizoLEX** Reagent by repetitive pipetting. Use 1 ml of the reagent per 5-10 × 10^6 of animal, plant or yeast cells, or per 1 × 10^7 bacterial cells. Washing cells before addition of **TrizoLEX** Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

Cells (totally), WBC pellet and Liquid materials:

Disruption/Homogenization by vortex in **TrizoLEX** reagent.

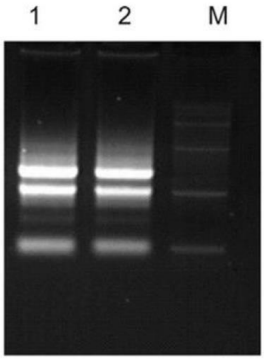
Tissue, Plant and Filamentous Fungi: Disruption by mortar and pestle liquid Nitrogen /Homogenization by syringe and needle in **TrizoLEX** reagent.

General procedure for RNA isolation

1. **PHASE SEPARATION:** Add 1ml ice-cold **TrizoLEX** solution to 2ml tube containing homogenized sample.
2. Vortex 15-30sec vigorously and incubate at room temperature for 5-15 min.
3. Add 0.2 ml of chloroform per 1 ml. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 4°C for 10 minutes (Do not vortex).
4. Centrifuge the samples no more than 12,000× *g* for 15 min at 2 to 8°C. Following centrifugation, the mixture separates into a lower yellow, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of **TrizoLEX** reagent used for homogenization.
5. **RNA PRECIPITATION:** Transfer the Aqueous phase (upper Phase) to new RNase-free 1.5ml tube; do not disturb the mid phase (Save the organic phase if isolation of DNA or protein is desired).
6. Precipitate the RNA by mixing equal volume of isopropanol. Use 0.5 ml of isopropyl alcohol per 1 ml of **TrizoLEX** reagent used for the initial homogenization. Mix by pipetting up and down gently, (Do not vortex.) Then incubate on ice for 15-30 min.
7. Centrifuge the mixture at 12,000× *g* at 2 to 8°Cfor 15min.
8. **RNA WASH**: Discard the supernatant and add 1ml of 75% Ethanol. Do not stir the precipitate, gentlely inverting the tube several times to wash the tube. Centrifuge at 12,000 x g for 2 min at 4 °C, discard the ethanol (**Optional**: repeat the step again).
9. **REDISSOLVING THE RNA**: Remove the supernatant and let the pellet to air dried briefly at room temperature for 5-10 min (Do not over dry the pellet and don't dry the RNA by centrifugation under vacuum either. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility). Dissolve pellet in 50µl of DEPC treated water and

incubate for 10 min at 55 to 60°C. Partially dissolved RNA samples have an A260/280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be redissolved in 100% formamide (deionized) and stored at -70°C.

Figure 1: Extraction RNA of Lettuce by TrizoLEX Reagent
Lane M: 15000 bp Marker
Lane 1 and 2: Lettuce RNA



Expected yields of RNA per mg of tissue or 1 × 106 cultured cells:

Type of tissue or cells	Expected yields
Liver and spleen	6-10 µg
Skeletal muscles and brain	1-1.5 µg
Placenta	1- 4 µg
Epithelial cells (1×10^6 cultured cells)	8-15 µg
Fibroblasts, (1×10^6 cultured cells)	5-7 µg

Troubleshooting

Problem	Comments
RNA degradation	-Tissues were not immediately processed or frozen after removal from the animal. Samples used for isolation, or isolated RNA preparations were stored at -5 to-20°C, instead of -60 to -70°C. -Cells dispersed by trypsin digestion. -Aqueous solutions or tubes were not RNase-free. -Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.
Low yield	-Incomplete homogenization or lysis of samples. -Final RNA pellet incompletely redissolved.
A260/A280 ratio <1.65	-RNA sample diluted in water instead of TE prior to spectrophotometric analysis. Low ionic strength and low pH solutions increase absorbance at 280 nm. -Sample homogenized in too small a reagent volume. -Following homogenization, samples were not stored at room temperature for 5 minutes. -The aqueous phase contaminated with the phenol phase. -Incomplete dissolution of the final RNA pellet**.**
DNA contamination	-Sample homogenized in too small a reagent volume. -Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.
Proteoglycan and polysaccharide contamination	The following modification of the RNA precipitation (RNA precipitation) removes these contaminating compounds from the isolated RNA. Add to the aqueous phase 0.25 ml of isopropanol followed by 0.25 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 ml of Reagent used for the homogenization. Mix the resulting solution, centrifuge and proceed with the isolation as described in the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in a soluble form.

Other products & services:

- ✓ Cloning and expression of different recombinant peptides
- ✓ Gene, Primer and peptide synthesizing
- ✓ Bioinformatics services
- ✓ Production of column based DNA extraction kits.
- ✓ Production of secondary antibodies (goat anti mouse, anti rabbit and anti human antibodies, HRP conjugated).
- ✓ PFU master mix
- ✓ Molecular grade buffers (TAE, TBE, RIPA and….)
- ✓ And ….

For more information visit us at “www.dnabiotech.ir”

More Products Launch Coming Soon!