DATA SHEET

TRIsure™

Catalog Numbers

TRIsure	BIO-38032	100ml
TRIsure	BIO-38033	200ml

Exp. Date: See vial

Batch No.: See vial

Store at Room Temperature

Storage and Stability:
TRIsure can be stored for 12 months at +4°C.

Shipping:
Dry/Blue Ice

Safety Precautions:
Toxic in contact with skin. Toxic if swallowed. Causes burns. Please refer to the material safety data sheet for information regarding hazards and safe handling practice.

Signal word : DANGER

Notes:
1. This product insert is a declaration of analysis at the time of manufacture.
2. Research Use Only.

Features
- Quick isolation of high-quality RNA
- Ready-to-use solution for a wide variety cells and tissues
- Cost-effective
- Convenient 1hr protocol
- Performs well with large or small amounts of tissue or cells

Applications
- Isolation of high quality RNA from diverse biological material, including animal and plant tissues rich in polysaccharides and proteoglycans
- Purified RNA is ideal for any downstream application such as RT-PCR or *in vitro* translation

Description

TRIsure is a ready-to-use reagent for the isolation of total RNA from cells and tissues. TRIsure maintains the integrity of the extracted RNA, while disrupting cells and subsequently dissolving cell components. The reagent combines a blend of phenol and other components.

The biological sample is homogenized or lysed in TRIsure and then separated into organic and aqueous phases. The RNA remains in the aqueous phase and is subsequently recovered by precipitation with isopropyl alcohol.

The isolated RNA is suitable for any downstream application such as RT-PCR, hybridization assays, or *in vitro* translation. 1ml of TRIsure is sufficient to isolate Total RNA from 1 x 107 cells or 100mg of tissue.

The expected yield of RNA from 1mg of tissue is:
- 2-5µg from mouse kidney
- 5-10µg from mouse liver

The expected yield of RNA from 1 x 106 cultured cells is:
- 8-15µg from epithelial cells
- 20-25µg from cell fibroblasts

Protocol for the Isolation of RNA using TRIsure

Reagents Required:
- Chloroform
- Isopropyl alcohol (chilled)
- 75% Ethanol (in DEPC-treated water)
- DEPC-treated water

1. Homogenization

Tissue:
Homogenize tissue samples in 1 ml of TRIsure per 50-100mg of tissue. For small quantities of tissue (1-10mg), add 800µl of TRIsure. For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed.
Plant tissue
Following homogenization, insoluble material is removed by centrifugation at 12,000 x g for 10 minutes at 2-8°C. Transfer the cleared homogenate to a fresh tube.
Cells Grown on Monolayer:
Lyse cells directly in a culture dish or flask by adding 1ml of TRIsure per 10cm2 growth area, pipette the cell lysate several times to ensure sufficient cell disruption.
Cells Grown in Suspension:
Pellet cells at 200 x g for 5 minutes at room temperature. Lyse cells with 1 ml of TRIsure per 5 x 106 cells and pass the lysate several times through a pipette tip. For small quantities of cells (102 -106), lyse cells in 800µl of TRIsure.
Note: At this stage, samples can be stored for at least one month at -60 to -70°C.

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2. Phase Separation

Incubate samples for 5 minutes at room temperature. Add 0.2 ml of chloroform per 1 ml of TRIsure used. Cap tubes securely and shake vigorously by hand for 15 seconds.
Incubate samples for 2-3 minutes at room temperature. Centrifuge samples at 12,000 x g for 15 minutes (or 2600 x g for 20-30 minutes) at 2-8°C. The sample will separate into a pale green, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase that contains the RNA.

3. RNA Precipitation

Transfer the aqueous phase very carefully, without disturbing the interphase to another tube. Precipitate the RNA by mixing with cold isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIsure used. Incubate samples for 10 minutes at room temperature then centrifuge at 12,000 x g for 10 minutes (or 2600 x g for 20-30 minutes) at 2-8°C.
Note: For small quantities of cells, RNase-free Glycogen Co-precipitant (BIO-37077) can be added to the aqueous phase before addition of isopropyl alcohol to aid RNA precipitation. Add 5-10□g of Glycogen per 800□l of TRIsure.

4. RNA Wash

Remove the supernatant. Wash the pellet once with 75% ethanol, adding at least 1 ml of ethanol per 1 ml of TRIsure used. Vortex samples and centrifuge at 7500 x g for 5 minutes at 2-8°C.

Note: At this stage, samples can be stored for one week at 2-8°C, or 12 months at -5 to -20°C.

5. Re-dissolving the RNA

Air-dry the pellet and dissolve in RNase free water (BIO-37080) or DEPCtreated Water (BIO-38030) by pipetting the solution up and down.

Incubate for 10 minutes at 55-60°C if necessary. Store RNA at -70°C.

Troubleshooting Guide

Problem	Possible Cause	Recommendation
Genomic DNA contamination*	Insufficient volume of TRIsure used	Ensure that 1 ml TRIsure per 10cm^2 area of cells or 5 x 10^6 cells is used. If problem persists, increase TRIsure volume by 1.5 x.
	Incomplete lysis or homogenization	Homogenize tissue thoroughly and centrifuge to remove insoluble material. Pipette resultant cell lysate up and down thoroughly until it becomes visually less viscous.
	Contamination of interphase layer during separation of the RNA-containing aqueous layer	Pipette off the aqueous phase very carefully. It is important that none of the white interphase is transferred into your RNA sample, so we recommend that you leave the lower part of the aqueous phase intact.
Low RNA yield	Loss of pellet	If starting sample is small, the RNA pellet may not be easily visualized after isopropyl alcohol precipitation, so care must be taken when removing the supernatant from the pellet.
	Incomplete lysis or homogeniza-tion	Homogenize tissue thoroughly and centrifuge to remove insoluble material. Pipette resultant cell lysate up and down thoroughly until it becomes visually less viscous.
	Incomplete solubilization of final RNA pellet	Ensure RNA pellet is completely dissolved in solution.
RNA degradation	RNase contamination	The protocol must be carried out carefully in a DNA-free, RNase-free environment. Ensure all pipettes, tips, tubes and work areas are free from RNases and wear gloves. Addition of RNase Inhibitor (BIO-65028) to the extracted RNA sample can help prevent degradation of the sample.

* If downstream applications could be affected by small amounts of DNA, we recommend an additional step of treating the RNA sample with RNase free DNase I

Associated Products

Product Name	Pack Size	Catalog No.
cDNA Synthesis Kit	100ml	BIO-65025
MyTaq™ One-Step RT-PCR Kit	25 Lanes	BIO-65033
MyTaq™ HS Mix	25 Lanes	BIO-25045
dNTP Set	10,000 Units	BIO-39025
SensiFAST™ SYBR Kit	1ml	BIO-98002

Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.