# TRIsure™

**Catalog Numbers**

| | | |
|---|---|---|
| TRIsure | BIO-38032 | 100 mL |
| TRIsure | BIO-38033 | 200 mL |

Batch No.: See vial

**Store at +4 °C**

meridian BIOSCIENCE™

**Storage and Stability:**
TRIsure is shipped at room temperature. For optimal performance, we recommend to store at +4 °C.

**Expiry:**
When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.

**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:**
For research or further manufacturing use only.

**Trademarks:**


## Features

- Quick isolation of high-quality total RNA, DNA and/or protein from a single sample
- Performs well with large or small amounts of tissue or cells
- Ready-to-use solution

## Applications

- Purified RNA is ideal for any downstream application such as RT-PCR, *in vitro* translation, northern blotting, RNase protection assays or dot blot hybridization
- Purified DNA can be used for PCR and Southern blotting
- Purified protein can be used for western blotting

## Description

TRIsure™ is a complete, ready-to-use reagent for the isolation of RNA, DNA and protein from cells and tissues. Using TRIsure, a biological sample is homogenized and lysed before being separated into three phases: an aqueous phase (upper), an organic phase (lower) and an interphase. The RNA is extracted from the aqueous phase by isopropyl alcohol precipitation. The highly effective RNase inhibitory property of TRIsure protects the integrity of the RNA during the lysis and results in the isolation of high-quality material. DNA is precipitated from the organic layer with ethanol. Protein is sequentially precipitated from the phenol-ethanol supernatant by isopropyl alcohol precipitation.

1 mL of TRIsure is sufficient to isolate RNA and DNA from 1 x $10^7$ cells or 100 mg of tissue. The table below presents typical yields of RNA and DNA from various starting materials.

| Starting material | Quantity | RNA | DNA |
|---|---|---|---|
| Mouse liver | 1 mg | 2-5 µg | 3-4 µg |
| Mouse kidney | 1 mg | 5-10 µg | 3-4 µg |
| Epithelial cells | 1 x $10^6$ cells | 8-15 µg | 5-7 µg |
| Fibroblast cells | 1 x $10^6$ cells | 20-25 µg | 5-7 µg |

## Protocol for the isolation of RNA using TRIsure

**Reagents required (not supplied):**

- Chloroform
- Isopropyl alcohol (chilled)
- 75% ethanol (in DEPC-treated water)
- DEPC-treated water or PCR water

### 1. Homogenization

*Tissue:*
Homogenize tissue samples in 1 mL of TRIsure per 50-100 mg of tissue. For small quantities of tissue (1-10 mg), add 800 µL 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 4 °C. Transfer the cleared homogenate to a fresh tube.

*Cells Grown on Monolayer:*
Lyse cells directly in a culture dish or flask by adding 1 mL of TRIsure per 10$cm^2$ 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 $10^6$ cells and pass the lysate several times through a pipette tip. For small quantities of cells ($10^2$ -$10^6$), lyse cells in 800 µL of TRIsure.

*Note: At this stage, samples can be stored for at least one month at -70 °C.*

### 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 3 minutes at room temperature. Centrifuge samples at 12,000 x g for 15 minutes (or 2600 x g for 30 minutes) at 4 °C. The sample will separate into a pale green, organic phase, an interphase, and a colorless upper aqueous phase.

The aqueous phase is removed for RNA extraction.

### 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 30 minutes) at 4 °C.

*Note: For small quantities of cells, RNase-free Co-precipitant Pink (BIO-37075) can be added to the aqueous phase before addition of isopropyl alcohol to aid visualizing RNA precipitation. Add 5-10 µg of Co-precipitant per 800 µL 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 4 °C.

*Note: At this stage, samples can be stored for one week at 4 °C, or 12 months at -20 °C.*

### 5. Re-dissolving the RNA

Air-dry the pellet and dissolve in PCR water or DEPC-treated water by pipetting the solution up and down. Incubate for 10 minutes at 60 °C if necessary. Store RNA at -70 °C.

## Protocol for the isolation of DNA using TRIsure

**Reagents required (not supplied):**

- 100% ethanol
- 0.1 M sodium citrate in 10% ethanol
- 75% ethanol
- 8 mM NaOH
- DEPC-treated water or PCR water

After homogenization and phase separation the upper aqueous phase is removed for optional RNA precipitation, leaving the interphase and the organic phase for sequential isolation of DNA and protein.

*Note: The interphase and organic phase can be stored overnight at 4 °C.*

### 1. DNA Precipitation

Remove any remaining aqueous phase overlying the interphase (step 3 of the RNA isolation protocol). Add 0.3 mL of 100% ethanol per 1 mL of TRIsure used, and mix samples by inversion. Leave samples at room temperature for 3 minutes, then centrifuge at 2000 x g for 5 minutes at 4 °C.

*Note: At this stage, samples can be stored for at least one month at 4 °C.*



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**2. DNA Wash**
Remove the supernatant to waste or retain for protein isolation. Wash the DNA pellet, with 1 mL of 0.1 M sodium citrate in 10% ethanol per 1 mL of TRIsure used, and mix for 30 minutes at room temperature. Centrifuge samples at 2000 x g for 5 minutes at 4 °C. Two washes are usually sufficient, however for large pellets containing >200 µg of DNA an additional wash may be necessary.

Following the wash steps, add 1.5 mL of 75% ethanol per 1 mL of TRIsure used. Mix for 20 minutes at room temperature, then centrifuge samples at 2000 x g for 5 minutes at 4 °C.

**3. Re-dissolving the DNA**
Air-dry the pellet for 15 minutes. Resuspend the pellet in 8 mM NaOH Remove any insoluble material by centrifugation at 12000 x g for 10 minutes and then transfer the supernatant to another tube.

*Note: Samples dissolved in 8 mM NaOH can be stored overnight at 4 °C. For long-term storage, adjust the pH to 7.5, and add EDTA to achieve a 1 mM concentration. Store at -20 °C.*

## Protocol for the isolation of protein using TRIsure

**Reagents required (not supplied):**

- Isopropyl alcohol
- 0.3 M Guanidine hydrochloride in 95% ethanol
- Ethanol
- 1% SDS

**1. Protein Precipitation**
To the retained supernatant (step 2 of the DNA isolation protocol) add 1.5 mL of isopropyl alcohol per 1 mL of TRIsure used. Mix samples for 10 minutes at room temperature then centrifuge at 12000 x g for 10 minutes at 4 °C.

**2. Protein Wash**
Remove supernatant and wash the protein pellet twice. To wash the protein pellet add 2 mL of 0.3 M guanidine hydrochloride in 95% ethanol per 1 mL of TRIsure used. Mix for 20 minutes at room temperature then centrifuge at 7500 x g for 5 minutes at 4 °C.

*Note: At this stage, samples can be stored for at least one month at 4 °C, or 12 months at -20 °C.*

Following the washing steps, add 2 mL of ethanol and vortex. Mix for 20 minutes at room temperature then centrifuge at 7500 x g for 5 minutes at 4 °C.

*Note: At this stage, samples can be stored for at least one month at 4 °C, or 12 months at -20 °C.*

**3. Re-dissolving the Protein**
Vacuum dry the protein pellet for 5-10 minutes. Dissolve in 1% SDS by pipetting up and down. For difficult samples incubate at 50 °C. Remove any insoluble material by centrifugation at 10000 x g for 10 minutes at 4 °C and then transfer the supernatant to another tube. Store protein at -20 °C.

**References:**
Chomczynski, P. and Sacchi, N. *Anal. Biochem.* **162:** 156-159. (1987)
Chomczynski, P. *BioTechniques* **15:** 532-537. (1993)

## Troubleshooting Guide

| Problem | Possible Cause | Recommendation |
|---|---|---|
| DNA contamination or RNA contamination | Interphase/organic phase pipetted up with aqueous phase | Do not attempt to draw off the entire aqueous layer after phase separation. |
| | Insufficient removal of the aqueous phase from the organic phase | Remove remnants of the aqueous phase prior to DNA precipitation. |
| | Insufficient wash of the DNA pellet | Make sure pellet is washed with 0.1 M sodium citrate in 10% ethanol. |
| Low RNA yield | Insufficient homogenisation or lysis of samples | Decrease the amount of starting material. Mince tissues into smaller pieces and make sure it is completely immersed in TRIsure for optimal lysis. |
| | Insufficient solubilization of RNA, DNA or protein pellet | Increase the solubilization by pipetting the sample repeatedly, heat the sample to 60 °C. |
| | Loss of pellet | If starting sample is small, the pellet may not be easily visualized after precipitation, so care must be taken when removing the supernatant from the pellet. |
| DNA is degraded, DNA is degraded or protein is degraded | Samples were not immediately processed or frozen after collection | Sample must be processed or frozen immediately after collection. |
| | Isolated RNA, DNA or protein preparations were stored at the incorrect temperature | Store RNA samples at -80 °C. Store DNA and protein samples at -20 °C. |
| | RNase contamination | Protocol must be carried out carefully in a DNA-free, RNase-free environment. Addition of RNase Inhibitor to the extracted RNA sample can help prevent degradation of the sample. |
| Low $A_{260/280}$ for RNA | Insufficient volume of TRIsure | Ensure that 1 mL TRIsure per 10 $cm^2$ area of cells or 5 x $10^6$ cells is used. If problem persists, increase TRIsure volume by 1.5 x. |
| | 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 $A_{260/280}$ for DNA | Phenol was not sufficiently removed from the DNA preparation | Wash the DNA pellet one additional time in 0.1 M sodium citrate in 10% ethanol. |

## Technical Support

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

Email: mbi.tech@meridianlifescience.com

## Associated Products

| Product Name | Pack Size | Catalog No. |
|---|---|---|
| SensiFAST™ cDNA Synthesis Kit | 50 Reactions | BIO-65053 |
| MyTaq™ HS Mix | 200 Reactions | BIO-25045 |
| SensiFAST™ SYBR® No-ROX One-Step Kit | 100 Reactions | BIO-72001 |

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