

# TRIzol™ LS Reagent and Phasemaker™ Tubes Complete System

Catalog Numbers A33252 and A33253

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**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

## Product information

Invitrogen™ TRIzol™ LS Reagent is a ready-to-use reagent, designed to isolate high quality total RNA from cell and tissue samples of human, animal, plant, yeast, or bacterial origin, within one hour. TRIzol™ LS Reagent is a monophasic solution of phenol, guanidine isothiocyanate, and other proprietary components which facilitate the isolation of a variety of RNA species of large or small molecular size. TRIzol™ LS Reagent maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization. TRIzol™ LS Reagent allows for simultaneous processing of a large number of samples, and is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (Chomczynski and Sacchi, 1987).

TRIzol™ LS Reagent allows to perform precipitation of RNA from a single sample (Chomczynski, 1993). After homogenizing the sample with TRIzol™ LS Reagent, chloroform is added, and the homogenate is allowed to separate into a clear upper aqueous layer (containing RNA), an interphase, and a red lower organic layer (containing the DNA and proteins). Phasemaker™ Tubes facilitate the transfer of the aqueous layer containing the RNA by creating a physical barrier between the upper and lower phases. RNA is precipitated from the aqueous layer with isopropanol. The precipitated RNA is washed to remove impurities, and then resuspended for use in downstream applications. Phasemaker™ Tubes are inert, heat stable, and do not interfere with standard RNA 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.

**IMPORTANT!** TRIzol™ LS Reagent is designed for processing liquid samples (blood and virus preparations, for example). Do not use TRIzol™ LS Reagent undiluted with solid samples. Processing solid samples with TRIzol™ LS Reagent results in decreased yield.

## Contents and storage

Contents	Cat. No. A33252 (100 reactions)	Cat. No. A33253 (200 reactions)	Storage
TRIzol™ LS Reagent	100 mL	200 mL	15–30°C
Phasemaker™ Tubes	100 tubes	200 tubes	

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). MLS: Fisher Scientific ([fisherscientific.com](http://fisherscientific.com)) or other major laboratory supplier.

Item	Source
<b>Equipment</b>	
Centrifuge and rotor capable of reaching 12,000 × <i>g</i> and 4°C	MLS
Water bath or heat block at 55–60°C	MLS
<b>Tubes</b>	
Polypropylene microcentrifuge tubes	MLS
<b>Reagents</b>	
Chloroform	MLS
Isopropanol	MLS
Ethanol, 75%	MLS
RNase-free water of 0.5% SDS	MLS
<i>(Optional)</i> RNase-free glycogen	MLS

## Input sample requirements

**IMPORTANT!** Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at –80°C or in liquid nitrogen until RNA isolation.

Sample type	Starting material per 0.75 mL of TRIzol™ LS Reagent
Biological fluids <sup>[1]</sup>	0.25 mL of biological sample

<sup>[1]</sup> Biological fluids with high levels of contamination material (whole blood, for instance) should be diluted 1:1 with RNase-free water.

## Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use cold TRIzol™ LS Reagent if the starting material contains high levels of RNase, such as spleen or pancreas samples.
- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified materials.
- Always use proper microbiological aseptic techniques when working with RNA.
- Use RNaseZap™ RNase Decontamination Solution (Cat. no. AM9780) to remove RNase contamination from work surfaces and non-disposable items such as centrifuges and pipettes used during purification.
- Always maintain a ratio of 3:1 between the volume of TRIzol™ LS Reagent and the sample.
- To facilitate isolation of RNA from small quantities of samples (<10<sup>6</sup> cells or <10 mg of tissue) or for sample volumes <0.25 mL, adjust the sample volume to 0.25 mL with RNase-free water.

## Isolate RNA

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- 1 Lyse samples and separate phases**
- Centrifuge the Phasemaker™ Tubes for 30 seconds at 12,000–16,000 × g prior to use.
  - Add 0.75 mL of TRIzol™ LS Reagent per 0.25 mL of sample volume.
  - Homogenize the sample by pipetting up and down several times.  
**Note:** The sample volume should not exceed 10% of the volume of TRIzol™ LS Reagent used for lysis. If the ratio is higher, add 50–100 µL of nuclease-free water to the lysate.
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- STOPPING POINT** Samples can be stored at 4°C overnight or at –20°C for up to a year.
- (Optional) If samples have a high fat content, centrifuge the lysate for 5 minutes at 12,000 × g at 4–10°C, then transfer the clear supernatant to a new tube.
  - Transfer the samples (0.1–1.3 mL) to the Phasemaker™ Tubes .
  - Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
  - Add 0.2 mL of chloroform per 0.75 mL of TRIzol™ LS Reagent used for lysis, then securely cap the tube. The total volume of the lysate cannot exceed 1.7 mL.
  - Shake vigorously by hand for 15 seconds.
  - Incubate for 2–15 minutes.
  - Centrifuge the sample for 5 minutes at 12,000–16,000 × g at 4°C.  
The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase. The Phasemaker™ Gel forms a barrier between the upper and lower phases.
  - Transfer the aqueous phase containing the RNA to a new tube.  
**IMPORTANT!** Avoid puncturing or touching the Phasemaker™ Gel with the pipette when removing the aqueous phase.
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- 2 Precipitate the RNA**
- Add 0.5 mL of isopropanol to the aqueous phase, per 0.75 mL of TRIzol™ LS Reagent used for lysis.
  - Incubate for 10 minutes.
  - Centrifuge for 10 minutes at 12,000 × g at 4°C.  
Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.
  - Discard the supernatant with a micropipettor.
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- 3 Wash the RNA**
- Resuspend the pellet in 1 mL of 75% ethanol per 0.75 mL of TRIzol™ LS Reagent used for lysis.  
**Note:** The RNA can be stored in 75% ethanol for at least 1 year at –20°C, or at least 1 week at 4°C.
  - Vortex the sample briefly, then centrifuge for 5 minutes at 7500 × g at 4°C.
  - Discard the supernatant with a micropipettor.
  - Vacuum or air dry the RNA pellet for 5–10 minutes.  
**IMPORTANT!** Do not dry the pellet by vacuum centrifuge. Do not let the RNA pellet dry, to ensure total solubilization of the RNA. Partially dissolved RNA samples have an  $A_{230/280}$  ratio <1.6.
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- 4 Solubilize the RNA**
- Resuspend the pellet in 20–50 µL of RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution by pipetting up and down.  
**IMPORTANT!** Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.
  - Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.  
Proceed to downstream applications, or store the RNA at –70°C.
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## 5 Determine the RNA yield

Determine the RNA yield using one of the following methods.

Method	Procedure
<p>Absorbance</p> <p>Absorbance at 260 nm provides total nucleic acid content, while absorbance at 280 nm determines sample purity. Since free nucleotides, RNA, ssDNA, and dsDNA absorb at 260 nm, they all contribute to the total absorbance of the sample.</p>	<ol style="list-style-type: none"> <li>Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm.</li> <li>Calculate the RNA concentration using the formula <math>A_{260} \times \text{dilution} \times 40 = \mu\text{g RNA/mL}</math>.</li> <li>Calculate the <math>A_{260}/A_{280}</math> ratio. A ratio of ~2 is considered pure.</li> </ol> <p>RNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectrophotometer. Refer to the instrument's instructions for more information.</p>
<p>Fluorescence</p> <p>Fluorescence selectively measures intact RNA, but does not measure protein or other contaminant present in the sample</p>	<ul style="list-style-type: none"> <li>Quantify RNA yield using the appropriate Qubit™ or Quant-iT™ RNA Assay Kit (Cat. Nos. Q32852, Q10210, Q33140, or Q10213). Refer to the kit's instructions for more information.</li> </ul>

**Table 1** Typical RNA ( $A_{260}/A_{280}$  of >1.8) yields from various starting materials

Starting material	Quantity	RNA yield
Human blood	250 $\mu\text{L}$	2.6–4.0 $\mu\text{g}$
	1 mL	15–20 $\mu\text{g}$
Human leukocytes	$7 \times 10^7$ cells	60–70 $\mu\text{g}$
	$7 \times 10^8$ cells	1109 $\mu\text{g}$

## Troubleshooting

Observation	Possible cause	Recommended action
A lower yield than expected is observed	The samples were incompletely homogenized or lysed.	Decrease the amount of starting material. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in TRIzol™ LS Reagent to achieve total lysis.
	The pellet was incompletely solubilized	Increase the solubilization rate by pipetting the sample repeatedly, and heat the sample to 50–60°C.
	The aqueous phase is incompletely removed.	Phasemaker™ Gel sits at the interphase, therefore little DNA will be present in the aqueous phase. Treat samples with DNase prior to any downstream applications. Make sure that all of the aqueous layer is transferred to a new tube.
The sample is degraded	Samples were not immediately processed or frozen after collection.	Sample must be processed or frozen immediately after collection.
	Sample preparations were stored at the incorrect temperature.	Store RNA samples at –60 to –70°C.
The RNA is contaminated	The pipette tip came in contact with the interphase/organic phase.	Phasemaker™ Gel sits at the interphase, therefore little DNA will be present in the aqueous phase. Treat samples with DNase prior to any downstream applications.
	The Phasemaker tube containing the lysate was centrifuged at room temperature.	Centrifuge the Phasemaker™ tube containing the lysate at 4°C. Higher temperatures increase the presence of DNA in the aqueous phase.
The RNA $A_{260}/A_{280}$ ratio is low	Sample was homogenized in an insufficient volume of TRIzol™ LS Reagent.	Add the appropriate amount of TRIzol™ LS Reagent for your sample type. Always maintain a ratio of 3:1 between the volume of TRIzol™ LS Reagent and the sample.
Phasemaker™ Gel does not appear uniform	The TRIzol™ LS Reagent to sample ratio is less than 3:1.	If the barrier is intact, proceed with protocol. If there appears to be a hole or space in the barrier, or the aqueous phase has colored hue, retrieve the sample and place in a new Phasemaker™ tube. Add chloroform and 50–100 $\mu\text{L}$ of nuclease-free water, then proceed according to protocol.

## Limited product warranty

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## References

- Chomczynski, P. 1993. *A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples*. *BioTechniques* 15, 532–537
- Chomczynski, P., and Sacchi, N. 1987 *Single Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction*. *Anal. Biochem.* 162, 156–159

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Revision	Date	Description
A.0	09 November 2016	New document

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