

## Product Information

### TRIzol LS Reagent

Catalog Number	Packaging Size
FP313	100 mL

#### Storage upon receipt:

- 2-8°C
- Protect from light

### Product Description

**TRIzol LS Reagent** is a ready-to-use reagent, designed to isolate high quality total RNA (as well as DNA and proteins) from a variety of liquid samples. **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.

**TRIzol LS Reagent** allows to perform sequential precipitation of RNA, DNA, and proteins from a single sample. 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 blue lower organic layer (containing the DNA and proteins). RNA is precipitated from the aqueous layer with isopropanol. DNA is precipitated from the interphase/organic layer with ethanol. Protein is precipitated from the phenol-ethanol supernatant by isopropanol precipitation. The precipitated RNA, DNA, or protein is washed to remove impurities, and then resuspended for use in downstream 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 some immunoprecipitation.

### Required materials not supplied

- Chloroform
- Isopropanol
- 75% Ethanol
- RNase-free water
- microcentrifuge tubes
- Centrifuge capable of reaching 12,000 × g and 4°C

### Input sample requirements

**Note:** 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	0.25 mL of biological sample

### Lyse samples and separate phases

1. Add 0.75 mL of TRIzol LS Reagent per 0.25 mL of sample volume.
  2. Homogenize the sample by pipetting up and down several times.
  3. (*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.
  4. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
  5. Add 0.2 mL of chloroform per 0.75 mL of TRIzol LS Reagent used for lysis, then securely cap the tube, and mix vigorously by hand for 15s.
  6. Incubate for 2-3 minutes.
  7. Centrifuge the sample for 15 minutes at 12,000 × g at 4°C.
- The mixture separates into a lower blue phenol-chloroform, and interphase, and a colorless upper aqueous phase.
8. Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.

### Isolate RNA

1. Precipitate the RNA
  - a. Add 0.5 mL of isopropanol to the aqueous phase, per 0.75 mL of TRIzol LS Reagent used for lysis.
  - b. Incubate for 10 minutes.
  - c. 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.

  - d. Discard the supernatant with a micropipettor.
2. Wash the RNA
  - a. 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.

  - b. Vortex the sample briefly, then centrifuge for 5 minutes at 7500 × g at 4°C.
  - c. Discard the supernatant with a micropipettor.
  - d. Vacuum or air dry the RNA pellet for 5-10 minutes.
3. Solubilize the RNA
  - a. 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.

**Note:** Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.

  - b. Incubate in a water bath or heat block set at 55-60°C for 10-15 minutes.

4. Determine the RNA yield by OD measurement
  - a. Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm.
  - b. Calculate the RNA concentration using the formula  $A_{260} \times \text{dilution} \times 40 = \mu\text{g RNA/mL}$ .

c. Calculate the  $A_{260}/A_{280}$  ratio. A ratio of ~2 is considered pure.

#### 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 $\mu\text{g}$
Human leukocytes	$5 \times 10^7$ cells	60-70 $\mu\text{g}$

#### Trouble shooting

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.
	The pellet was incompletely solubilized	Increase the solubilization rate by pipetting the sample repeatedly, and heat the sample to 50–60°C.
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. Store DNA and protein samples at -20°C.
The RNA is contaminated	The interphase/organic phase is pipetted up with the aqueous phase.	Do not attempt to draw off the entire aqueous layer after phase separation.
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.
	The organic phase is incompletely removed.	Do not attempt to draw off the entire aqueous layer after phase separation.