

TRIzol™ LS Reagent

Catalog Numbers 10296010 and 10296028

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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.

Product information

Invitrogen™ TRIzol™ LS Reagent is a ready-to-use reagent, designed to isolate high quality total RNA (as well as DNA and proteins) 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 sequential precipitation of RNA, DNA, and proteins 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). 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.

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.

TRIzol™ LS Reagent can also be used with Phasemaker™ Tubes to isolate RNA. Refer to *TRIzol™ LS Reagent and Phasemaker™ Tubes Complete System User Guide* (MAN0016164) for the full protocol.

Contents and storage

Contents	Cat. No. 10296010 (100 reactions)	Cat. No. 10296028 (200 reactions)	Storage
TRIzol™ LS Reagent	100 mL	200 mL	15–30°C

Required materials not supplied

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

Table 1 Materials required for RNA, DNA, and protein isolation

Item	Source
Equipment	
Centrifuge and rotor capable of reaching 12,000 × <i>g</i> and 4°C	MLS
Tubes	
Polypropylene microcentrifuge tubes	MLS
Reagents	
Chloroform	MLS

Table 2 Materials required for RNA isolation

Item	Source
Equipment	
Water bath or heat block at 55–60°C	MLS
Reagents	
Isopropanol	MLS
Ethanol, 75%	MLS
RNase-free water of 0.5% SDS	MLS

Table 3 Materials required for DNA isolation

Item	Source
Reagents	
Ethanol, 100%	MLS
Ethanol, 75%	MLS
0.1 M sodium citrate in 10% ethanol	MLS
8 mM NaOH	MLS
HEPES	MLS

Table 4 Materials required for protein isolation

Item	Source
Equipment	
(Optional) Dialysis membranes	MLS
Reagents	
Isopropanol	MLS
Ethanol, 100%	MLS
0.3 M Guanidine hydrochloride in 95% ethanol	MLS
1% SDS	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 ^[1]	0.25 mL of biological sample

^[1] 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 (<10⁶ 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.

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.
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 red phenol-chloroform, and interphase, and a colorless upper aqueous phase.
8. Transfer the aqueous phase containing the RNA to a new tube.
9. Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.

IMPORTANT! Avoid transferring any of the interphase or organic layer into the pipette when removing the aqueous phase.

Proceed directly to “Isolate RNA” on page 2.

Save the interphase and organic phase if you want to isolate DNA or protein. See “Isolate DNA” on page 3 or “Isolate proteins” on page 4 for detailed procedures. The organic phase can be stored at 4°C overnight.

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.

Note: The sample volume should not exceed 10% of the volume of TRIzol™ LS Reagent used for lysis.

STOPPING POINT Samples can be stored at 4°C overnight or at –20°C for up to a year.

Isolate RNA

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- | | | |
|----------|----------------------------|--|
| 1 | Precipitate the RNA | <ol style="list-style-type: none">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. |
| <hr/> | | |
| 2 | Wash the RNA | <ol style="list-style-type: none">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. <hr/> <p>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.</p> <hr/> |
| 3 | Solubilize the RNA | <ol style="list-style-type: none">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. <hr/>IMPORTANT! Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions. <hr/>b. 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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4 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"> 1. Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm. 2. Calculate the RNA concentration using the formula $A_{260} \times \text{dilution} \times 40 = \mu\text{g RNA/mL}$. 3. Calculate the A_{260}/A_{280} ratio. A ratio of ~2 is considered pure. <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"> • 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.

Table 5 Typical RNA (A_{260}/A_{280} of >1.8) yields from various starting materials

Starting material	Quantity	RNA yield
Human blood	250 μL	2.6–4.0 μg
	1 mL	15–20 μg
Human leukocytes	7×10^7 cells	60–70 μg
	7×10^8 cells	1109 μg

Isolate DNA

Isolate DNA from the interphase and the lower phenol-chloroform phase saved from “Lyse samples and separate phases” on page 2.

1 Precipitate the DNA

- Remove any remaining aqueous phase overlying the interphase.
This is critical for the quality of the isolated DNA.
- Add 0.3 mL of 100% ethanol per 0.75 mL of TRIzol™ LS Reagent used for lysis.
- Cap the tube, mix by inverting the tube several times.
- Incubate for 2–3 minutes.
- Centrifuge for 5 minutes at $2000 \times g$ at 4°C to pellet the DNA.
- Transfer the phenol-ethanol supernatant to a new tube.
The supernatant is used for protein isolation (see “Isolate proteins” on page 40, if needed, and can be stored at –70°C for several months).

2 Wash the DNA

- Resuspend the pellet in 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 0.75 mL of TRIzol™ LS Reagent used for lysis.
- Incubate for 30 minutes, mixing occasionally by gentle inversion.
Note: The DNA can be stored in sodium citrate/ethanol for at least 2 hours.
- Centrifuge for 5 minutes at $2000 \times g$ at 4°C.
- Discard the supernatant with a micropipettor.
- Repeat step 2a–step 2d once.
Note: Repeat step 2a–step 2d twice for large DNA pellets (>200 μg).
- Resuspend the pellet in 1.5–2 mL of 75% ethanol per 0.75 mL of TRIzol™ LS Reagent used for lysis.
- Incubate for 10–20 minutes, mixing occasionally by gentle inversion.
Note: The DNA can be stored in 75% ethanol at several months at 4°C.
- Centrifuge for 5 minutes at $2000 \times g$ at 4°C.
- Discard the supernatant with a micropipettor.
- Vacuum or air dry the DNA pellet for 5–10 minutes.

IMPORTANT! Do not dry the pellet by vacuum centrifuge.

3 Solubilize the DNA

- Resuspend the pellet in 0.3–0.6 mL of 8 mM NaOH by pipetting up and down.
Note: We recommend resuspending the DNA in a mild base because isolated DNA does not resuspend well in water or Tris buffer.
- Centrifuge for 10 minutes at $12,000 \times g$ at 4°C to remove insoluble materials.
- Transfer the supernatant to a new tube, then adjust pH as needed with HEPES.

Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C, adjust the pH to 7–8 with HEPES and add 1 mM EDTA.

4 Determine the DNA yield

Determine the DNA yield using one of the following methods.

Method	Procedure
Absorbance 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.	<ol style="list-style-type: none">1. Dilute sample in water or buffer (pH >7.5), then measure absorbance at 260 nm and 280 nm.2. Calculate the DNA concentration using the formula $A_{260} \times \text{dilution} \times 50 = \mu\text{g DNA/mL}$.3. Calculate the A_{260}/A_{280} ratio. A ratio of ~1.8 is considered pure. DNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectrophotometer. Refer to the instrument's instructions for more information.
Fluorescence Fluorescence selectively measures intact DNA, but does not measure protein or other contaminant present in the sample	<ul style="list-style-type: none">• Quantify dsDNA yield using the appropriate Qubit™ or Quant-iT™ dsDNA Assay Kit (Cat. Nos. Q32850, Q32851, Q33120, or Q33130). Refer to the kit's instructions for more information.

Table 6 Typical DNA ($A_{260}/280$ of 1.6–1.8) yields from human leukocytes

Starting material	Quantity	DNA yield
Human leukocytes	7×10^8 cells	1.3 mg

Isolate proteins

Isolate the proteins from the phenol-ethanol supernatant saved from “Precipitate the DNA” on page 3 using either “Precipitate the proteins” on page 4 or “Dialyze the proteins” on page 4.

- 1 Precipitate the proteins**
 - a. Add 1.5 mL of isopropanol to the phenol-ethanol supernatant per 0.75 mL of TRIzol™ LS Reagent used for lysis.
 - b. Incubate for 10 minutes.
 - c. Centrifuge for 10 minutes at $12,000 \times g$ at 4°C to pellet the proteins.
 - d. Discard the supernatant with a micropipettor.

- 2 Wash the proteins**
 - a. Prepare a wash solution consisting of 0.3 M guanidine hydrochloride in 95% ethanol.
 - b. Resuspend the pellet in 2 mL of wash solution per 0.75 mL of TRIzol™ LS Reagent used for lysis.
 - c. Incubate for 20 minutes.
Note: The proteins can be stored in wash solution for at least 1 month at 4°C or for at least 1 year at –20°C.
 - d. Centrifuge for 5 minutes at $7500 \times g$ at 4°C.
 - e. Discard the supernatant with a micropipettor.
 - f. Repeat step 2b–step 2e twice.
 - g. Add 2 mL of 100% ethanol, then mix by vortexing briefly.
 - h. Incubate for 20 minutes.
 - i. Centrifuge for 5 minutes at $7500 \times g$ at 4°C.
 - j. Discard the supernatant with a micropipettor.
 - k. Air dry the protein pellet for 5–10 minutes.

IMPORTANT! Do not dry the pellet by vacuum centrifuge.

- 3 Solubilize the proteins**
 - a. Resuspend the pellet in 200 μL of 1% SDS by pipetting up and down.
Note: To ensure complete resuspension of the pellet, we recommend that you incubate the sample at 50°C in a water bath or heat block.
 - b. Centrifuge for 10 minutes at $10,000 \times g$ at 4°C to remove insoluble materials.
 - c. Transfer the supernatant to a new tube.Proceed directly to downstream applications, or store the sample at –20°C.

- 4 Determine the protein yield**
 - Measure protein concentration by Bradford assay.
Note: SDS concentration must be <0.1%.

Dialyze the proteins

1. Load the phenol-ethanol supernatant into the dialysis membrane.
Note: The phenol-ethanol solution can dissolve some types of dialysis membranes (cellulose ester, for example). Test dialysis tubing with the membrane to assess compatibility before starting.
2. Dialyze the sample against 3 changes of 0.1% SDS at 4°C. Make the first change of solution after 16 hours, the second change 4 hours later (at 20 hours), and the final change 2 hours later (at 22 hours).
Note: A SDS concentration of at least 0.1% is required to resolubilize the proteins from the pellet. If desired, the SDS can be diluted after solubilization.
3. Centrifuge the dialysate for 10 minutes at $10,000 \times g$ at 4°C.

4. Transfer the supernatant containing the proteins to a new tube.
5. (Optional) Solubilize the pellet by adding 100 μ L of 1% SDS and 100 μ L of 8 M urea.

Proceed directly to downstream applications, or store the sample at -20°C .

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 $50\text{--}60^{\circ}\text{C}$.
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 or DNA 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 aqueous phase is incompletely removed.	Remove remnants of the aqueous phase prior to DNA precipitation.
	The DNA pellet is insufficiently washed with 0.1 M sodium citrate in 10% ethanol	Make sure pellet is washed with 0.1 M sodium citrate in 10% ethanol.
The RNA $A_{260/280}$ 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.
The DNA $A_{260/280}$ ratio is low	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.

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

The information in this guide is subject to change without notice.

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Revision history: Pub. No. MAN0000806

Revision	Date	Description
A.0	09 November 2016	Added references to Phasemaker™ Tubes .
—	15 November 2010	Baseline for revision.

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Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

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