TRIzol™ Reagent

Experimental protocol for DNA isolation

Catalog Numbers 15596026 and 15596018

Pub. No. MAN0016385 **Rev.** A.0

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™ 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™ 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™ 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™ Reagent allows for simultaneous processing of a large number of samples, and is an improvement to the single-step RNA isolation method developed by Chomcynski and Sacchi (Chomczynski and Sacchi, 1987).

TRIzol™ Reagent allows to perform sequential precipitation of RNA, DNA, and proteins from a single sample (Chomczynski, 1993). After homogenizing the sample with TRIzol™ 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.

For RNA and protein isolation, refer to *TRIzol™ Reagent User Guide* (Pub. No. MAN0001271).

TRIzol™ Reagent can also be used with Phasemaker™ Tubes to isolate RNA. Refer to *TRIzol™ Reagent and Phasemaker™ Tubes Complete System User Guide* (Pub. No. MAN0016163) for the full protocol.

Contents and storage

Contents	Cat. No. 15596026 (100 reactions)	Cat. No. 15596018 (200 reactions)	Storage
TRIzol™ 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 all isolations

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

Table 2 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

Input sample requirements

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

Sample type	Starting material per 1 mL of TRIzol™ Reagent
Tissues[1]	50–100 mg of tissue
Cells grown in monolayer	1×10^5–1×10^7 cells grown in monolayer in a 3.5–cm culture dish (10 cm^2)
Cells grown in suspension	5–10 $\times 10^6$ cells from animal, plant, or yeasty origin or 1 $\times 10^7$ cells of bacterial origin

[1] Fresh tissues or tissues stored in RNA*later*™ Stabilization Solution (Cat. No. AM7020).

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use cold TRIzol™ 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 RNase*Zap*™ 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.

Lyse samples and separate phases

1. Lyse and homogenize samples in TRIzol™ Reagent according to your starting material.
   - **Tissues:**

     Add 1 mL of TRIzol™ Reagent per 50–100 mg of tissue to the sample and homogenize using a homogenizer.
   - **Cell grown in monolayer:**
     a. Remove growth media.
     b. Add 0.3–0.4 mL of TRIzol™ Reagent per 1×10^5—10^7 cells directly to the culture dish to lyse the cells.
     c. Pipet the lysate up and down several times to homogenize.
   - **Cells grown in suspension:**
     a. Pellet the cells by centrifugation and discard the supernatant.
     b. Add 0.75 mL of TRIzol™ Reagent per 0.25 mL of sample (5–10×10^6 cells from animal, plant, or yeasty origin or 1×10^7 cells of bacterial origin) to the pellet.

     Note: Do not wash cells before addition of TRIzol™ Reagent to avoid mRNA degradation.
     c. Pipet the lysate up and down several times to homogenize.

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

   STOPPING POINT Samples can be stored at 4°C overnight or at –20°C for up to a year.
2. *(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.
3. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
4. Add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent used for lysis, then securely cap the tube.
5. Incubate for 2–3 minutes.
6. 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.
7. Discard the aqueous phase containing the RNA, then proceed directly to the next section with the interphase containing the DNA.

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

a. Remove any remaining aqueous phase overlying the interphase.

   This is critical for the quality of the isolated DNA.
b. Add 0.3 mL of 100% ethanol per 1 mL of TRIzol™ Reagent used for lysis.
c. Cap the tube, mix by inverting the tube several times.
d. Incubate for 2–3 minutes.
e. Centrifuge for 5 minutes at 2000 × *g* at 4°C to pellet the DNA.
f. Transfer the phenol-ethanol supernatant to a new tube.

   The supernatant is used for protein isolation [refer to *TRIzol™ Reagent User Guide* (Pub. No. MAN0001271)], if needed, and can be stored at –70°C for several months.

2 Wash the DNA

a. Resuspend the pellet in 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 1 mL of TRIzol™ Reagent used for lysis.
b. Incubate for 30 minutes, mixing occasionally by gentle inversion.

   Note: The DNA can be stored in sodium citrate/ethanol for at least 2 hours.
c. Centrifuge for 5 minutes at 2000 × *g* at 4°C.
d. Discard the supernatant with a micropipettor.
e. Repeat step 2a–step 2d once.

   Note: Repeat step 2a–step 2d twice for large DNA pellets (>200 µg).
f. Resuspend the pellet in 1.5–2 mL of 75% ethanol per 1 mL of TRIzol™ Reagent used for lysis.
g. 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.
h. Centrifuge for 5 minutes at 2000 × *g* at 4°C.
i. Discard the supernatant with a micropipettor.
j. Vacuum or air dry the DNA pellet for 5–10 minutes.

   IMPORTANT! Do not dry the pellet by vacuum centrifuge.

3 Solubilize the DNA

a. Resuspend the pellet in 0.3–0.6 mL of 8 mM NaOH by pipetting up and down.

   Note: We recommend resuspending the DNA is a mild base because isolated DNA does not resuspend well in water or Tris buffer.
b. Centrifuge for 10 minutes at 12,000 × *g* at 4°C to remove insoluble materials.
c. 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 dsDNS absorb at 260 nm, they all contribute to the total absorbance of the sample.	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 A260 × dilution × 50 = µg DNA/mL. 3. Calculate the A260/A280 ratio. A ratio of ~1.8 is considered pure. DNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectophotometer. 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	• Quantify dsDNA yield using the appropriate Qubit™ or Quant-iT™ ds DNA Assay Kit (Cat. Nos. Q32850, Q32851, Q33120, or Q33130). Refer to the kit's instructions for more information.

Table 3 Typical DNA ($A_{260/280}$ of 1.6–1.8) yields from various starting materials

Starting material	Quantity	DNA yield
Fibroblasts	1×10^6 cells	5–7 µg
Cultured cells, mammal	1×10^6 cells	5–7 µg
Skeletal muscles and brain	1 mg	2–3 µg
Placenta	1 mg	2–3 µg
Liver	1 mg	3–4 µg
Kidney	1 mg	3–4 µg

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™ 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 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 DNA is contaminated	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 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.

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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

Hummon, A. B., Lim S. R., Difilippantonio, M. J., and Ried, T. 2007 *Isolation and solubilization of proteins after TRIzol® extraction of RNA and DNA from patient material following prolonged storage.* BioTechniques *42*, 467-472

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Revision	Date	Description
A.0	08 December 2016	New document

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