# TRIzol™ Reagent and Phasemaker™ Tubes Complete System

**Catalog Numbers** A33250 and A33251

**Pub. No.** MAN0016163 **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 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 precipitation of RNA 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). 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.

## Contents and storage

| Contents | Cat. No. A33250 (100 reactions) | Cat. No. A33251 (200 reactions) | Storage |
|---|---|---|---|
| TRIzol™ 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**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

| Item | Source |
|---|---|
| **Equipment** | |
| Centrifuge and rotor capable of reaching 12,000 × *g* and 4°C | MLS |
| Water bath or heat block at 55–60°C | MLS |
| **Tubes** | |
| Polypropylene microcentrifuge tubes | MLS |
| **Reagents** | |
| Chloroform | MLS |
| Isopropanol | MLS |
| Ethanol, 75% | MLS |
| RNase-free water of 0.5% SDS | MLS |
| *(Optional)* 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 | Volume of TRIzol™ Reagent | Starting material |
|---|---|---|
| Tissues[1] | 1 mL | 50–100 mg of tissue |
| Cells grown in monolayer | 0.5 mL | $5 \times 10^3$–$1 \times 10^6$ cells grown in monolayer in a 3.5–cm culture dish (10 $cm^2$) |
| Cells grown in suspension | 0.75 mL | 5–10 × $10^6$ cells from animal, plant, or yeasty origin or 1 × $10^7$ 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.
- Always maintain a ratio of 10:1 between the volume of TRIzol™ Reagent and the sample.

## Isolate RNA

### 1 Lyse samples and separate phases

a. Centrifuge the Phasemaker™ Tubes for 30 seconds at 12,000–16,000 × *g* prior to use.
b. 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 sample, or per 0.25 mL of tissue suspension, then homogenize using a homogenizer.
   - **Cell grown in monolayer:**
     a. Remove growth media.
     b. Add 0.5 mL of TRIzol™ Reagent per $5 \times 10^3$—$1 \times 10^6$ 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 ($5–10 \times 10^6$ cells from animal, plant, or yeasty origin or $1 \times 10^7$ 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. If the ratio is higher, add 50–100 µL of nuclease-free water to the lysate.

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

c. *(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.
d. Transfer the samples (0.1–1.4 mL) to the Phasemaker™ Tubes .
e. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
f. Add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent used for lysis, then securely cap the tube.

   The total volume of the lysate cannot exceed 1.7 mL.
g. Shake vigorously by hand for 15 seconds.
h. Incubate for 2–3 minutes.
i. 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.
j. Transfer the aqueous phase (~50% of the starting TRIzol™ Reagent) containing the RNA to a new tube.

**IMPORTANT!** Avoid puncturing or touching the Phasemaker™ Gel with the pipette when removing the aqueous phase.

### 2 Precipitate the RNA

a. *(Optional)* If the starting sample is small (<$10^6$ cells or <10 mg of tissue), add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase.

   **Note:** The glycogen is co-precipitated with the RNA, but does not interfere with subsequent applications.
b. Add 0.5 mL of isopropanol to the aqueous phase, per 1 mL of TRIzol™ Reagent used for lysis.
c. Incubate for 10 minutes.
d. 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.
e. Discard the supernatant with a micropipettor.

### 3 Wash the RNA

a. Resuspend the pellet in 1 mL of 75% ethanol per 1 mL of TRIzol™ 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.

**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.

### 4 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.

**IMPORTANT!** 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.

Proceed to downstream applications, or store the RNA at –70°C.

## 5 Determine the RNA yield

Determine the RNA yield using one of the following methods.

| Method | Procedure |
|---|---|
| Absorbance<br>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 RNase-free water, then measure absorbance at 260 nm and 280 nm.<br>2. Calculate the RNA concentration using the formula A260 × dilution × 40 = µg RNA/mL.<br>3. Calculate the A260/A280 ratio.<br>A ratio of ~2 is considered pure.<br>RNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectophotometer. Refer to the instrument's instructions for more information. |
| Fluorescence<br>Fluorescence selectively measures intact RNA, but does not measure protein or other contaminant present in the sample | • Quantify RNA yield using the appropriate Qubit™ or Quant-iT™ RNA Assay Kit (Cat. Nos. Q32852, Q10210, Q33140, or Q10213).<br>Refer to the kit's instructions for more information. |

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

| Starting material | Quantity | RNA yield |
|---|---|---|
| Epithelial cells | $1 \times 10^6$ cells | 8–15 µg |
| New tobacco leaf | — | 73 µg |
| Fibroblasts | $1 \times 10^6$ cells | 5–7 µg |
| Skeletal muscles and brain | 1 mg | 1–1.5 µg |
| Placenta | 1 mg | 1–4 µg |
| Liver | 1 mg | 6–10 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 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.<br>Higher temperatures increase the presence of DNA in the aqueous phase. |
| The RNA $A_{260/280}$ ratio is low | Sample was homogenized in an insufficient volume of TRIzol™ Reagent. | Add the appropriate amount of TRIzol™ Reagent for your sample type. Always maintain a ratio of 10:1 between the volume of TRIzol™ Reagent and the sample. |
| Phasemaker™ Gel does not appear uniform | The TRIzol™ Reagent to sample ratio is less than 10:1. | 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 µL 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 |
|---|---|---|
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