# TRIzol™ Plus RNA Purification Kit and Phasemaker™ Tubes Complete System

**Catalog Numbers** A33254

**Pub. No.** MAN0016165 **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™ Plus RNA Purification Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a wide variety of samples, including animal and plant cells and tissue, bacteria, and yeast. The kit utilizes the strong lysis capability of TRIzol™ Reagent, followed by a convenient and time-saving silica-cartridge purification protocol from the PureLink™ RNA Mini Kit, to purify ultrapure total RNA within an hour, even from difficult samples such as fibrous tissue.

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 or lysis.

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.

## Contents and storage

Reagents provided in the kit are sufficient for 50 RNA isolations.

**Table 1** TRIzol™ Plus RNA Purification Kit and Phasemaker™ Tubes Complete System Cat. No. A33254

| Contents | Amount | Storage |
|---|---|---|
| TRIzol™ Reagent | 100 mL | 15–30°C |
| Phasemaker™ Tubes , 2 mL | 100 tubes | |
| PureLink™ RNA Mini Kit[1] | 1 kit | |

[1] See Table 2 for kit contents.

**Table 2** PureLink™ RNA Mini Kit Cat. No. 12183018A

| Contents | Amount | Storage |
|---|---|---|
| Spin cartridges (with collection tubes) | 50 cartridges | 15–30°C |
| Collection tubes | 50 tubes | |
| Wash Buffer I | 50 mL | |
| Wash Buffer II | 15 mL | |
| RNase-free water | 15.5 mL | |
| Recovery tubes | 50 tubes | |
| Lysis Buffer[1] | 125 mL | |

[1] Not used in this protocol.

## 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 |
| Homogenizer, or equivalent | 12183026 |
| **Tubes and tips** | |
| 1.5-mL RNase-free microcentrifuge tubes | MLS |
| RNase-free pipette tips | MLS |
| **Reagents** | |
| Chloroform, or 4-bromoanisole | MLS |
| Ethanol, molecular grade | 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 1 mL of TRIzol™ Reagent |
|---|---|
| Tissues[1] | 50–100 mg of tissue |
| Cells grown in monolayer | $2 \times 10^5$–$2 \times 10^7$ cells grown in monolayer in a 3.5–cm culture dish (10 $cm^2$) |
| Cells grown in suspension | 10–20 $\times 10^6$ cells from animal, plant, or yeasty origin or 2 $\times 10^7$ 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 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™ Plus RNA Purification Kit and the mass of the sample.

- The maximum RNA binding capacity of the spin cartridge is ~1 mg. If you are processing samples that contain more than 1 mg of total RNA, divide the sample into aliquots containing <1 mg total RNA for each spin cartridge used.
- Use clear polypropylene disposable tubes when working with <2 mL volumes of TRIzol™ Reagent. For larger volumes, use glass or polypropylene tubes, and ensure that the tubes can withstand centrifugation at 12,000 × *g* with TRIzol™ Reagent and chloroform. Do not use tubes that leak or crack.

## Before first use of the kit

1. Add 60 mL of 96–100% ethanol to Wash Buffer II, then mix thoroughly.
2. Check the box on the Wash Buffer II label to indicate that ethanol was added, then store at room temperature.

## 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 to the sample and homogenize using a homogenizer.
   - **Cell grown in monolayer:**
     a. Remove growth media.
     b. Add 1 mL of TRIzol™ Reagent per $2 \times 10^5$—$10^7$ 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 ($10–20 \times 10^6$ cells from animal, plant, or yeasty origin or $2 \times 10^7$ 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.

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 or 50 µL of 4-bromoanisole 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 volume of the starting lysate) containing the RNA to a new tube.

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

k. Add an equal volume of 70% ethanol, then mix well by vortexing.
l. Invert the tube to disperse any visible precipitate that may form after adding ethanol.

### 2 Bind the RNA to the membrane

a. Transfer up to 700 µL of the sample to a spin cartridge (with collection tube).
b. Centrifuge at 12,000 × *g* for 15 seconds.
c. Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
d. Repeat step 2a–step 2c until the entire sample has been processed.
e. *(Optional)* If your downstream application required DNA-free total RNA, perform on-column DNase treatment (see *PureLink™ RNA Mini Kit User Guide* (Pub. No. MAN0000406).

### 3 Wash the RNA on the membrane

a. Add 700 µL of Wash Buffer I to the spin cartridge.
b. Centrifuge at 12,000 × *g* for 15 seconds.
c. Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
d. Add 500 µL of Wash Buffer II to the spin cartridge.
e. Centrifuge at 12,000 × *g* for 15 seconds.
f. Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
g. Repeat step 3d-step 3f once.

### 4 Elute the RNA

a. Centrifuge at 12,000 × *g* for 1 minute to dry the membrane.
b. Discard the collection tube, then insert the spin cartridge into a recovery tube.

**4** Elute the RNA *(continued)*

c. Add 30 µL-3 × 100 µL (3 sequential elutions with 100 µL each) of RNase-free water to the center of the spin cartridge.

**Note:** If you are performing sequential elutions, collect all eluates in the same tube.

d. Incubate for 1 minute.

e. Centrifuge at >12,000 × *g* for 2 minutes.

f. Discard the spin cartridge.

The recovery tube contains the purified total RNA.

- Store the purified RNA on ice if used within a few hours. For long-term storage, store the purified RNA at –80°C.
- If highly pure RNA without genomic DNA contamination is required, perform DNase I treatment after purification (see *PureLink™ RNA Mini Kit User Guide* (Pub. No. MAN0000406).
- You can determine the quality and quantity of the purified RNA using UV absorbance at 260 nm or with the Quant-iT™ RNA Assay Kit (Cat. No. Q33140).

## Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| A lower RNA yield is observed | The samples were incompletely homogenized or lysed. | Use the appropriate method for lysate preparation based on your starting material (see "Input sample requirements" on page 1). |
| | | 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 starting material is of poor quality. | Use fresh samples immediately after collection or freeze sample at –80°C or in liquid nitrogen until further use. |
| | The spin cartridge is clogged. | Clear lysate and remove any particulate or viscous material by centrifugation, and use only the supernatant for subsequent loading onto the spin cartridge. |
| | Ethanol was not added to Wash Buffer II . | See "Before first use of the kit" on page 2. |
| | The elution conditions are incorrect. | Add RNase-free water (30 µL-3 × 100 µL) and incubate for 1 minute before centrifugation.<br>To recover more RNA, use up to 3 sequential elutions of 100 µL each. |
| The RNA is degraded | The RNA is contaminated with RNase. | Follow "Procedural guidelines" on page 1 to prevent RNase contamination. |
| | The samples were improperly handled from collection until lysis. | 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. |
| Downstream reactions are inhibited | Ethanol is present in the purified RNA. | The spin cartridge membrane has to be dry before eluting the RNA, as traces of ethanol from Wash Buffer II can inhibit downstream enzymatic reactions.<br>1. Discard Wash Buffer II flow-through.<br>2. Insert the spin cartridge into the collection tube.<br>3. Centrifuge for 1–2 minutes at 12,000 × *g* to completely dry the membrane.<br>4. Proceed with RNA elution. |
| | Salts are present in the purified RNA. | Always wash with Wash Buffer I before washing with Wash Buffer II. |
| The RNA $A_{260/280}$ ratio is low | The sample was diluted in water. | Use 10 mM Tris-HCl (pH 7.5) to dilute the samples for absorbance measurements. |
| Phasemaker™ Gel does not appear uniform | The TRIzol™ Plus RNA Purification Kit 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. |

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| Revision | Date | Description |
|---|---|---|
| A.0 | 09 November 2016 | New document |


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