ambion | RNA
by life technologies

TRIzol® Plus RNA Purification Kit

Catalog Number: 12183-555 **Quantity: 50 preps** **Store at room temperature**

Contents and Storage

The components of the TRIzol® Plus RNA Purification Kit are shipped at room temperature. Upon receipt, store all components at room temperature. The components included with the kit are listed in the table below. Sufficient reagents are provided to perform 50 total RNA isolations.

Components	Quantity
TRIzol® Reagent	100 mL
PureLink™ RNA Mini Kit Contents:	
Lysis Buffer	125 mL
Wash Buffer I	50 mL
Wash Buffer II	15 mL
RNase–Free Water	15.5 mL
Spin Cartridges (with collection tubes)	50 each
Collection Tubes	50 each
Recovery Tubes	50 each

About the Kit

The 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, while disrupting cells and dissolving cell components, and also provides an immediate and highly effective inhibition of RNase activity during sample homogenization or lysis.

The combination of TRIzol® Reagent followed by silica-cartridge purification of RNA is the recommended RNA purification method in the gene expression industry. The TRIzol® Plus RNA Purification Kit provides the reagents and an optimized protocol to purify total RNA for gene expression studies using an industry recommended method.

System Overview

To isolate purified RNA from your sample using the TRIzol® Plus RNA Purification Kit, the sample is first lysed with TRIzol® Reagent, according to the lysate preparation protocol provided. The addition of chloroform to your sample, followed by centrifugation separates the solution into an upper aqueous phase containing RNA and a lower phenol-containing organic phase. The upper aqueous phase is transferred to a new tube, followed by ethanol addition and centrifugation. The sample is then transferred to the PureLink™ RNA Mini Kit Spin Cartridge containing a clear silica–based membrane to which the RNA binds during purification. The RNA is washed to remove contaminants and the purified total RNA is then eluted in RNase-Free Water (Tris Buffer, pH 7.5 may also be used) and is suitable for use in a variety of downstream applications including sensitive gene expression studies such as microarray analysis or real time quantitative RT-PCR (qRT-PCR).

PureLink™ RNA Mini Kit Specifications

Cartridge Binding Capacity:	~1 mg nucleic acid
Cartridge Reservoir Capacity:	700 µL
Wash Tube Capacity:	2.0 mL
Centrifuge Compatibility:	Capable of centrifuging >12,000 × *g*
Elution Volume:	30 µL–3 × 100 µL (3 sequential elutions with 100 µL each)

Notes

- This manual provides instructions for purifying total RNA from samples using TRIzol® Reagent to prepare lysates and after phase separation, the RNA is purified using the PureLink™ RNA Mini Kit. If you wish to use other protocols for RNA purification, refer to the TRIzol® Reagent manual or the PureLink™ RNA Mini Kit manual, available at **www.invitrogen.com**.
- The TRIzol® Plus RNA Purification Kit utilizes the TRIzol® Reagent for sample lysis. The Lysis Buffer included with the kit is not used in this protocol. However, if you wish to prepare samples using the Lysis Buffer, refer to the PureLink™ RNA Mini Kit manual available from **www.invitrogen.com**.
- If your downstream applications require DNA-free total RNA, protocols for on-column PureLink™ DNase treatment during RNA purification or after purification are provided in the PureLink™ RNA Mini Kit manual, available from **www.invitrogen.com**.

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Caution

- TRIzol® Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Avoid direct contact with TRIzol® Reagent, as direct contact of skin, eyes, or respiratory tract with TRIzol® Reagent may cause chemical burns to the exposed area. When working with TRIzol® Reagent, **always** work in a fume hood. Refer to the TRIzol® Reagent product insert for more details. Contact your Environmental Heath and Safety (EH&S) department for proper work and disposal guidelines.
- Lysis Buffer and Wash Buffer I contain guanidine isothiocyanate (an irritant). This chemical is harmful when in contact with the skin, or inhaled, or ingested. **Do not** add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidinium isothiocyanate, as reactive compounds and toxic gases are formed.
- Solutions containing ethanol are considered flammable. Use appropriate precautions when using this chemical.
- For your protection, always wear a laboratory coat, gloves, and safety glasses when handling these chemicals. Dispose of the buffers and chemicals in appropriate waste containers.

General Guidelines

- The maximum RNA binding capacity of the PureLink™ RNA Mini Kit 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 disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free 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 material.
- Always use proper microbiological aseptic techniques when working with RNA.
- Use clear polypropylene disposable tubes when working with <2 mL volumes of TRIzol® Reagent. For larger volumes, use glass (Corex) 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.
- Use RNase *AWAY*® Reagent (Cat. no. 10328-011) to remove RNase contamination from work surfaces and non–disposable items such as centrifuges and pipettes used during purification.

Materials Needed

- Starting material (fresh or frozen tissue, or cells)
- TRIzol® Reagent and PureLink™ RNA Mini Kit (included)
- Chloroform or 4–Bromoanisole
- 96–100% ethanol and 70% ethanol (in RNase-free water)
- Microcentrifuge capable of centrifuging at 12,000 × *g*
- Homogenizer (Cat. no: 12183-026) or Rotor-Stator homogenizer
- 1.5 mL RNase-free microcentrifuge tubes and RNase-free pipette tips

Preparing Wash Buffer II with Ethanol

Before beginning lysis, add 60 mL 96–100% ethanol to Wash Buffer II. Check the box on the Wash Buffer II label to indicate that ethanol was added. Store Wash Buffer II with ethanol at room temperature.

Lysate Preparation with TRIzol® Reagent

Use TRIzol® Reagent to prepare lysates from various sample types as described below.

Tissues

Homogenize tissue samples in 1 mL TRIzol® Reagent per 50–100 mg tissue using a tissue homogenizer or rotor-stator. The sample volume should not exceed 10% of the volume of TRIzol® Reagent used for homogenization.

Adherent Cells

Lyse cells directly in a culture dish by adding 1 mL of TRIzol® Reagent to the dish and passing the cell lysate several times through a pipette tip. The amount of TRIzol® Reagent required is based on the culture dish area (1 mL per 10 cm^2) and not on the number of cells present.

Suspension Cells

Harvest cells and pellet cells by centrifugation. Use 1 mL of the TRIzol® Reagent per 5–10 × 10^6 animal, plant, or yeast cells, or per 1 × 10^7 bacterial cells. Lyse cells by repetitive pipetting up and down. Do not wash cells before addition of TRIzol® Reagent to avoid any mRNA degradation. Disruption of some yeast and bacterial cells may require a homogenizer.

Phase Separation

Following cell or tissue lysis (previous page), isolate the RNA as described below:

1. Incubate the lysate with TRIzol® Reagent (previous page) at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. Add 0.2 mL chloroform or 50 µL 4-Bromoanisole per 1 mL TRIzol® Reagent used. Shake the tube vigorously by hand for 15 seconds**.**

   Note: Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA or perform a DNase-digestion step during RNA purification or after purification Refer to the PureLink™ RNA Mini Kit manual, available from **www.invitrogen.com**.
3. Incubate at room temperature for 2–3 minutes.
4. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4°C.

   Note: After centrifugation, the mixture separates into a lower, red phenol–chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is ~600 µL.
5. Transfer ~600 µL of the colorless, upper phase **containing the RNA** to a fresh RNase-free tube.
6. Add an equal volume of 70% ethanol to obtain a final ethanol concentration of 35%. Mix well by vortexing.
7. Invert the tube to disperse any visible precipitate that may form after adding ethanol. Proceed to **Binding, Washing and Elution**, below.

Binding, Washing and Elution

1. Transfer up to 700 µL of sample (prepared as described above) to a Spin Cartridge (with a Collection Tube).
2. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow–through and reinsert the Spin Cartridge into the same Collection Tube.
3. **Repeat** Steps 1–2 until the entire sample has been processed.

   ***Optional*:** If your downstream application requires DNA-free total RNA, proceed to On-Column PureLink™ DNase Treatment During RNA Purification at this time (see the PureLink™ RNA Mini Kit manual, available from **www.invitrogen.com**, for details).
4. Add 700 µL Wash Buffer I to the Spin Cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the Collection Tube. Insert the Spin Cartridge into a new Collection Tube.
5. Add 500 µL Wash Buffer II with ethanol to the Spin Cartridge.
6. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.
7. Repeat Steps 5–6 **once.**
8. Centrifuge the Spin Cartridge and Collection Tube at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
9. Add 30 µL–3 × 100 µL (3 sequential elutions with 100 µL each) RNase-Free Water to the center of the Spin Cartridge (refer to the PureLink™ RNA Mini Kit manual for more details, available from **www.invitrogen.com**).
10. Incubate at room temperature for 1 minute.
11. Centrifuge the Spin Cartridge with the Recovery Tube for 2 minutes at ≥12,000 × *g* at room temperature. Discard the Spin Cartridge. *The recovery tube contains the purified total RNA.*

    Note: If you are performing sequential elutions, collect all elutes into the same tube.

Storage and Downstream Applications of Purified RNA

Store the purified RNA on ice if used within a few hours. For long-term storage, store the purified RNA at –80°C.

You may use the purified total RNA for qRT-PCR, northern blotting, nuclease protection assays, RNA amplification for microarray analysis, or any desired downstream application.

If highly pure RNA without genomic DNA contamination is required, perform DNase I treatment after purification (refer to the PureLink™ RNA Mini Kit manual available from **www.invitrogen.com** for details).

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).

Accessory Products

A large selection of products for RT-PCR, qRT-PCR, microarray analysis, and reverse transcription is available separately from Invitrogen. For more information, visit **www.invitrogen.com** or contact Technical Support.

Troubleshooting

Problem	Cause	Solution
Low RNA yield	Incomplete lysis and homogenization	• Use the appropriate method for lysate preparation based on your starting material as described on page 2. • Decrease the amount of starting material used. • Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the TRIzol® Reagent to achieve optimal lysis.
	Poor quality of starting material	The yield and quality of RNA isolated depends on the type and age of the starting material. Use fresh sample Immediately after harvesting, process the fresh sample or freeze the sample at –80ºC or in liquid nitrogen.
	Clogged RNA Spin Cartridge	Clear homogenate and remove any particulate or viscous material by centrifugation, and use only the supernatant for subsequent loading onto the Spin Cartridge.
	Ethanol not added to Wash Buffer II	Add ethanol to Wash Buffer II before use (page 2).
	Incorrect elution conditions	Add RNase-Free Water (30 µL–3 ×100 µL) and perform incubation for 1 minute before centrifugation. To recover more RNA, be sure to use up to 3 sequential elutions of 100 µL each (3 × 100 µL) Elution Buffer (refer to protocol on page 3).
RNA degraded	RNA contaminated with RNase	Follow the guidelines on page 2 to prevent RNase contamination.
	Improper handling of sample from harvest until lysis	If you are not processing your tissue samples immediately after harvest, quick-freeze tissue immediately after harvesting and store at –80°C or in liquid nitrogen. Keep the samples frozen until TRIzol® Reagent is added. Perform the lysis quickly after adding TRIzol® Reagent.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA	Traces of ethanol from the Wash Buffer II can inhibit downstream enzymatic reactions. Discard Wash Buffer II flow through. Place the Spin Cartridge into the Recovery Tube and centrifuge at 12,000 × *g* for 1–2 minutes to completely dry the cartridge.
	Presence of salt in purified RNA	Use the correct order of Wash Buffers for washing. Always wash with Wash Buffer I followed by Wash Buffer II.
Low A_{260}/A_{280} ratio	Sample was diluted in water	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.

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