Luna Nanotech

TotaZOL™ Total RNA Purification Kit

Product Manual

#NK057-5	5 preps
#NK057-50	50 preps
#NK057-200	200 preps

Manual Version 1.0
December 2025

Luna Nanotech Inc.
85 Citizen Court, Unit 13
Markham, ON, Canada
L6G 1A8

Email: support@lunanano.com
Phone: 1-800-474-4055
Web: www.lunanano.com

Table of Contents

Product Description 3

Contents 4

Storage 4

Additional Materials and Equipment Required 4

Important Notes 5

Input sample requirements 5

Protocol 6

Troubleshooting 8

Safety 11

Product Description

The TotaZOL™ Total RNA Purification Kit offers a fast, dependable, and straightforward approach for extracting high-quality total RNA from a broad range of sample types, including animal and plant cells and tissues, as well as bacteria and yeast. This kit combines the powerful cell-lysis action of TotaZOL™ Total RNA Purification Reagent with a streamlined silica-cartridge purification process from the PuroSPIN™ Total RNA Purification Kit #NK051-50, #NK051-250, enabling the isolation of highly pure total RNA in less than one hour, even from challenging samples such as fibrous tissues.

TotaZOL™ Total RNA Purification Reagent is a single-phase mixture containing phenol, guanidine isothiocyanate, and proprietary components that support the recovery of RNA molecules of both large and small sizes. It preserves RNA integrity by effectively inhibiting RNase activity while simultaneously breaking down cells and solubilizing cellular components during homogenization or lysis.

Contents

COMPONENT	#NK057-5 (5 reactions)	#NK057-50 (50 reactions)	#NK057-200 (200 reactions)
TotaZOL™ Total RNA Purification Reagent	10 mL	100 mL	2 x 200 mL
PuroSPIN™ MINI Spin Columns with collection tubes	5	50	200
Wash Buffer WB-TR1	10 mL	40 mL	160 mL
Wash Buffer WB-R2 *	10 mL *	120 mL *	2 x 225 mL *
Nuclease-Free Water	1 mL	20 mL	80 mL

* After addition of 96 – 100% Ethanol

Storage

All components of the kit can be stored at 18 - 25 °C for at least 1 year

Additional Materials Required

- 96 - 100% Ethanol
- 70% Ethanol
- Chloroform
- 10 mM Tris-HCl, pH 7.5
- 1.5 and/or 2.0 mL nuclease-free microcentrifuge tubes
- Microcentrifuge (wit 12,000 x g capability) and 4 °C)
- Pipettes and sterile nuclease-free pipette tips
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment (gloves, lab coat, goggles)

Important Notes

BEFORE USE:

- Add 96-100% Ethanol to the **Wash Buffer WB-R2**:
  - **#NK057-5** (5 preps): add **7.5 mL** of Ethanol
  - **#NK057-50** (50 preps): add **90 mL** of Ethanol
  - **#NK057-200** (200 preps): add **168 mL** of Ethanol to each bottle

Please take time to read this manual thoroughly before beginning to ensure you are familiar with all procedures. Prepare all necessary materials in advance to avoid RNA degradation.

- Always wear gloves when handling RNA to reduce the risk of RNase contamination.
- Use only sterile, disposable plasticware and pipettes dedicated to RNA work to prevent RNase cross-contamination.
- RNA remains protected from RNase activity while in TotaZOL™ Total RNA Purification Reagent. However, once removed, ensure that any reusable glassware or plasticware is RNase-free.
- Work quickly, but carefully.
- Use only DEPC-treated buffers: add DEPC to 0.1% final concentration, incubate at 37 °C for 2 hours, then autoclave at 121 °C. Do not add DEPC to Tris buffers; instead, prepare these with DEPC-treated water.
- Prepare all materials in advance.

Input sample requirements

Sample type	Starting material per 1 mL of TotaZOL Total RNA Purification Reagent
Tissues	50 - 100 mg
Cells grown in mono layer	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 yeast origin or 1 $\times 10^7$ cells of bacterial origin

Protocol

Step	Procedure
1	Homogenization and lysis of samples. Choose according to your starting material. • **Tissues:** Add 1 mL of **TotaZOL™ Total RNA Purification Reagent** per 50 - 100 mg of tissue. Homogenize using mechanical homogenizer. *Note: Alternately, grind tissue in liquid nitrogen with a mortar and pestle; transfer powder to a clean 1.5 mL microcentrifuge tube. If unavailable, homogenize tissue directly in the tube with a disposable 1.5 mL pestle.* • **Cell grown in monolayer:** Add 1 mL of **TotaZOL™ Total RNA Purification Reagent** per 1 x 10^5—1 × 10^7 cells directly to a 3.5 cm diameter culture plate and pipette to lyse the cells. *Note: It is critical to add sufficient amount of TotaZOL™ Total RNA Purification Reagent to the cells in order to avoid contaminations of extracted RNA with genomic DNA. If cell lysate is too viscous when aspirate with a pipette, add more Totazol™ Total RNA Purification Reagent.* • **Cell grown in suspension:** Add 1 mL of **TotaZOL™ Total RNA Purification Reagent** per 5-10 x 10^6 of animal, yeast, plant, or 1 x 10^7 bacterial cells. Pipette up and down to homogenize. *Note: It strongly advised to use mechanical or enzymatic homogenization for plant, fungal and yeast samples.*
2	*(Optional)* For samples with high fat content: centrifuge the lysate for 5 min at 12,000 x g at 4°C. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.
3	Incubate the sample for 5 minutes at room temperature to allow complete dissociation of the nucleoproteins complex.
4	Add 0.2 mL of **chloroform** per 1 mL of **TotaZOL™ Total RNA Purification Reagent** used for lysis. Cap the tube securely and shake vigorously for 15 seconds.
5	Incubate for 2-3 minutes at room temperature.

6	Centrifuge the sample for 15 minutes at 12,000 x g at 4°C.
7	After centrifugation the mixture separates into an upper colorless aqueous phase containing RNA, thin layer of white interphase and lower reddish phenol-chloroform phase. Carefully transfer around 600 µL aqueous phase to a new 1.5 mL microcentrifuge tube.
8	Add an equal volume of **70% Ethanol**, then mix well by inverting the tube 6-8 times.
9	Add 700 µL of sample to a new P**uroSPIN™ MINI Spin Column**. Centrifuge the column at 12,000 x g for 1 min. Discard the flow-through. Repeat until all sample is loaded to the column.
10	Add 700 µL of **Wash Buffer WB-TR1** to the column. Centrifuge the column at 12,000 x g for 1 min. Discard the flow-through.
11	Add 700 µL of **Wash Buffer WB-R2** (*with ethanol added*). Centrifuge the column at 12,000 x g for 1 min. Discard the flow-through
12	Repeat *Step 11* with another 700 µL of **Wash Buffer WB-R2**.
13	Centrifuge the column at 12,000 × g for 1 minute to dry the membrane.
14	Transfer the **PuroSPIN™ MINI Spin Column** into a new DNase-free 1.5 mL microcentrifuge tube.
15	Pipette 100 µL of **Nuclease-free water** onto the center of the column. Incubate for 2 min. Centrifuge at 12,000 x g for 1 min to elute the RNA. Keep the flow-through that contains RNA and discard the column.
16	Store purified RNA at –80°C.

Troubleshooting

Problem	Possible Causes and Solutions
Low RNA yield	**Insufficient amount of TotaZOL™ Total RNA Purification Reagent was used for lysis.** Add the indicated amount TotaZOL™ Total RNA Purification Reagent to the sample as indicated in *Step 1* of the Protocol. **The samples were incompletely homogenized or lysed** Follow homogenization method for specific sample type outlined in Step 1 of the Protocol. **Amount of starting amount was exceeded** Do not exceed amount of starting material as indicated in section "Input Sample Requirements" on Page 5 of the manual as it may clog the PuroSPIN™ MINI Spin Column. **70% Ethanol was not added to the lysate** Make sure to add 70 % Ethanol to the filtered lysate in Step 8 of the protocol. **Ethanol was not added to Wash Buffer WB-R2** Add the indicated amount of 96-100% Ethanol to the Wash buffer before use.
Degraded RNA	**Old or frozen tissue.** Use only fresh tissues. Avoid using frozen tissues. **Materials or reagent were contaminated with RNases** It is crucial to keep materials and reagents free of RNAses.

Genomic DNA	**Aqueous phase was contaminated with phenol-chloroform phase.** When transferring aqueous phase containing RNA avoid carrying organic phenol-chloroform phase that contains genomic DNA. **Insufficient amount of TotaZOL™ Total RNA Purification Reagent was used.** Use indicated amount of TotaZOL™ Total RNA Purification Reagent. Inadequate TotaZOL™ Total RNA Purification Reagent may cause poor separation of DNA nucleoproteins complex from aqueous RNA.
Low A260/A280 & A260/A230 ratios	**Insufficient TotaZOL™ Total RNA Purification Reagent during homogenization** Too little TotaZOL™ Total RNA Purification Reagent was used. In future preparations, decrease the amount of starting material and/or increase both the volume of TotaZOL™ Total RNA Purification Reagent and the homogenization time. **Phenol contamination in the aqueous phase** Phenol was carried over into the aqueous phase. When collecting the aqueous layer, avoid disturbing the interphase or organic phase. **Omission of the post-homogenization incubation** The sample was not incubated for 5 minutes after homogenization (Step 3 of the Protocol). Incubate the sample at room temperature (15–25 °C) for 5 minutes, as specified in the protocol, to ensure proper dissociation of nucleoprotein complexes.

	Inappropriate diluent for A260/A280 measurement Water was used to dilute the RNA. Instead, use 10 mM Tris·Cl, pH 7.5, for accurate absorbance measurements.
Downstream reactions are inhibited	**Ethanol is present in the purified RNA.** After washing the column with Wash Buffer WB-R2, dry the membrane as indicated in the protocol. **Purified RNA contains residual salts.** Make sure to Wash the column 2X with the indicated amount of Wash Buffer WB-R2.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
TotaZOL™ Total RNA Purification Reagent		Contains ***phenol*** and ***guanidine thyocyanite,*** • Harmful if swallowed. • Irritating to eyes and skin. • Do not breathe vapors and fumes. • Wear suitable protective clothing and gloves. • In case of contact with eyes immediately wash with plenty of water and seek medical advice. • If swallowed, seek medical advice and contact poison control center.

This product is intended solely for research use only. Full material safety data sheet document is available at www.lunanano.com.

For further information about this and other products please visit our website at www.lunanano.com.

Learn more at lunanano.com/dna-rna-purification-products

Luna Nanotech Inc.
85 Citizen Court, Unit 13
Markham, ON, Canada
L6G 1A8

Email: support@lunanano.com
Phone: 1-800-474-4055
Web: www.lunanano.com

Luna Nanotech
Nano for Bio