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INSTRUCTION MANUAL

Direct-zol™ RNA Miniprep Plus

Catalog Nos. R2070T, R2070, R2071, R2072, & R2073

Highlights

- Quick, spin column purification of high-quality (DNA-free) total RNA **directly** from TRIzol®, TRI Reagent® and other acid-guanidinium-phenol based reagents (RNAzol®, QIAzol®, TriPure™, TriSure™, etc.).
- Bypasses phase separation and precipitation procedures, for non-biased recovery of miRNA.

Contents

| | |
|---|---|
| Product Contents | 1 |
| Specifications | 1 |
| Product Description | 2 |
| Buffer Preparation | 3 |
| Protocols | |
| Cells, Tissue, Tough-to-Lyse Samples | 3 |
| Biological Liquids, Reaction Clean-up | 4 |
| Appendices | 5 |
| Ordering Information | 6 |

Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please call 1-888-882-9682.

Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

* TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

Product Contents

| Direct-zol™ RNA MiniPrep Plus Kit Size (Preps) | R2070 (50) | R2071 (50) | R2072 (200) | R2073 (200) | R2070T (10) |
|--|------------|------------|-------------|-------------|-------------|
| TRI Reagent® | - | 50 ml | - | 200 ml | - |
| Direct-zol™ RNA PreWash ¹ (concentrate) | 40 ml | 40 ml | 160 ml | 160 ml | 10 ml |
| RNA Wash Buffer ² (concentrate) | 12 ml | 12 ml | 48 ml | 48 ml | 16 ml |
| DNase I ³ (lyophilized) | 1 | 1 | 4 | 4 | 1 |
| DNA Digestion Buffer | 4 ml | 4 ml | 16 ml | 16 ml | 0.8 ml |
| DNase/RNase-Free Water | 10 ml | 10 ml | 2x 30 ml | 2x 30 ml | 1 ml |
| Zymo-Spin™ IICG Columns | 50 | 50 | 200 | 200 | 10 |
| Collection Tubes | 100 | 100 | 400 | 400 | 20 |
| Instruction Manual | 1 | 1 | 1 | 1 | 1 |

Storage Temperature - Store all kit components (*i.e.*, buffers, columns) at room temperature. TRI Reagent® is provided with catalog numbers **R2071** and **R2073** only.

- Before use, add 10 ml and 40 ml ethanol (95-100%) to the 40 ml and 160 ml **Direct-zol™ RNA PreWash** concentrate, respectively. Direct-zol RNA PreWash included with R2070T is supplied ready-to-use and does not require the addition of ethanol prior to use.
- Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate before use. RNA Wash Buffer included with R2070T is supplied ready-to-use and does not require the addition of ethanol prior to use.
- Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial prior to use. Store frozen aliquots.

Specifications

- Sample Sources** – Any sample stored and preserved in TRI Reagent®, TRIzol® or similar* (animal cells, tissue, bacteria, yeast, biological fluids, and *in vitro* processed RNA (*e.g.*, transcription products, DNase-treated or labeled RNA)).
- Sample Inactivation** – TRI Reagent® (provided with R2071, R2073 only) inhibits RNase activity and inactivates viruses and other infectious agents.
- RNA Size** – RNAs ≥17 nucleotides.
- RNA Purity** – $A_{260}/A_{280} >1.8$, $A_{260}/A_{230} >1.8$. Complete removal of DNA can be accomplished using an *in-column* DNase I digestion (page 4).
- RNA Recovery** – The RNA binding capacity of the **Zymo-Spin™ IICG Column** is ~100 µg.
- Compatibility** – TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and similar *acid-guanidinium-phenol* based reagents can be used in place of TRI Reagent®.
Also, compatible with samples in TRI Reagent® that contain chloroform, 1-bromo-3-chloropropane (BCP), or 4-bromoanisole (BAN), the aqueous phase of phase-separated samples (page 5), and samples stored in *RNAlater™* (page 5).
- RNA Storage** – RNA eluted with **DNase/RNase-Free Water** (provided) can be stored at ≤-70 °C. The addition of RNase inhibitors is highly recommended for prolonged storage.
- Equipment Needed** – Microcentrifuge.

This product is for research use only and not intended for use in diagnostic procedures. Follow applicable federal, state, and local regulations for phenol waste disposal.

™Trademarks of Zymo Research Corporation. Other trademarks: TRI Reagent®, TRIzol® and RNAzol® (Molecular Research Center, Inc.), QIAzol® (Qiagen GmbH), TriPure™ (Roche, Inc.), TriSure™ (Bioline Ltd.), RNAlater® (Ambion, Inc.), Bioanalyzer (Agilent Technologies, Inc.).

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Product Description



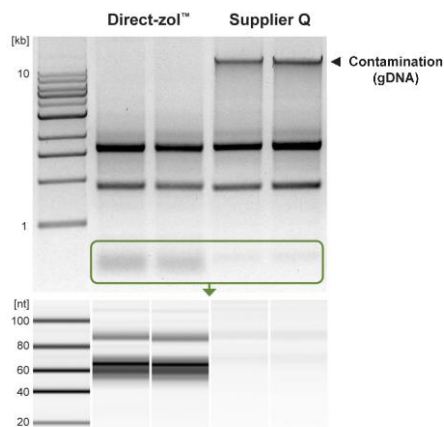
The **Direct-zol™ RNA MiniPrep Plus** provides a streamlined method for the purification of up to 100 µg (per prep) of high-quality RNA *directly* from samples in TRI Reagent® or similar¹. Total RNA, including small RNAs (17-200 nt), is effectively isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, biological liquids, *etc.*).

Isolation of RNA by conventional phase separation was shown² to selectively enrich for some species of miRNA, leading to bias in downstream analysis. The **Direct-zol™** method assures unbiased recovery of small RNAs including miRNA (see below).

The procedure is easy. Simply apply a prepared sample in TRI Reagent® directly to the **Zymo-Spin™ IICG Column** and then spin, wash, and elute the RNA. No phase separation, precipitation, or post-purification steps are necessary. The eluted RNA is high quality and suitable for subsequent molecular manipulation and analysis (including RT-PCR, transcription profiling, hybridization, sequencing *etc.*).

The entire procedure typically takes only 7 minutes.

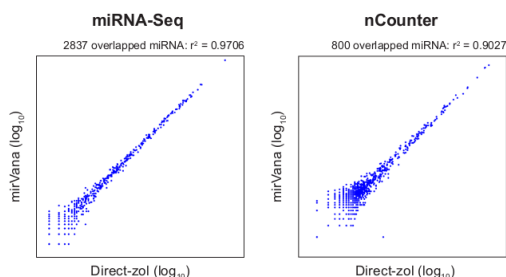
Efficient Recovery of Small & Large RNAs



(Top) High quality broad size-range DNA-free RNA is purified from human epithelial cells using the Direct-zol™ procedure compared to a preparation from Supplier Q (1% agarose/TAE gel).

(Bottom) Small RNAs are efficiently recovered with the Direct-zol™ procedure. However, this is not the case with Supplier Q's prep (Bioanalyzer, Small RNA Chip).

Non-biased miRNA Recovery



Micro-RNA isolation using Direct-zol™ RNA kits is not biased. The data show RNA purified from TRIzol® samples using the Direct-zol™ RNA MiniPrep compared to a method known to be unbiased (mirVana™, Ambion). Micro-RNA analysis was performed using miRNA-seq (MiSeq®, Illumina) and direct hybridization assay (nCounter®, Nanostring).

For **assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Note:

¹ TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

² Kim et al (2012) Molecular Cell 46(6):893-895

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Ensure RNA isolation is performed in an RNase-free environment.

Unless specified otherwise, all steps should be performed at room temperature.

Notes:

¹ RNAzol[®], QIAzol[®], TriPure[™], TriSure[™] and all other *acid-guanidinium-phenol* reagents.

² ZR BashingBead Lysis Tubes (2.0 mm), S6002 and FastPrep[®]-24, S6005.

Buffer Preparation

- ✓ Add 10 ml or 40 ml ethanol (95-100%) to the 40 ml or 160 ml **Direct-zol™ RNA PreWash** concentrate, respectively.
- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.

Protocol

This protocol consists of two parts: (I) Sample Preparation and (II) RNA Purification.

The following guidelines are provided for processing various sample types in TRI Reagent[®], TRIzol[®] or similar¹ *acid-guanidinium-phenol* reagents prior to spin column purification of the RNA.

RNA yield can vary with sample types, organism, quality and treatment of the starting material. To ensure complete lysis and homogenization of a sample, use a sufficient amount of TRI Reagent[®] or similar. For detailed processing information, refer to the TRI Reagent[®] product manual (or manufacturer's instructions for the reagent used).

(I) Sample Preparation

All centrifugation steps should be performed at 10,000-16,000 x *g* for 1 minute.

Cells

Lyse cells* directly in a culture dish** or resuspend pelleted cells in an appropriate volume (see table below) of TRI Reagent[®] and mix thoroughly. Proceed to RNA Purification (page 4).

| Animal | Bacterial | Yeast | Add TRI Reagent [®] |
|-------------------|-------------------|-------------------|------------------------------|
| ≤ 10 ⁵ | - | - | 100 μl |
| ≤ 10 ⁶ | ≤ 10 ⁸ | ≤ 10 ⁷ | 300 μl |
| ≤ 10 ⁷ | ≤ 10 ⁹ | ≤ 10 ⁸ | ≥ 600 μl |

* For cell suspensions, add 3 volumes of TRI Reagent[®] to 1 volume of cell suspension.

** For direct lysis in a dish, add 100 μl for each cm² of culture surface area.

Tissue

Lyse tissue sample in an appropriate volume (see table below) of TRI Reagent[®] and homogenize. To remove particulate debris from homogenized tissue, centrifuge and transfer the supernatant into an RNase-free tube. Proceed to RNA Purification (page 4).

Note: Sample should not exceed 10% of the TRI Reagent[®] volume used for homogenization.

| Animal | | Plant | Add TRI Reagent [®] |
|--|--|--|------------------------------|
| high yield (spleen, liver, etc.) | low yield (brain, heart, muscle, kidney, lung, etc.) | (root, leaves, stem, meristem, callus, etc.) | ≥ 600 μl |
| ≤ 20 mg | ≤ 50 mg | ≤ 200 mg | |

Tough-to-Lyse Samples (Cells and Tissue)

Tough-to-lyse samples (*i.e.*, gram-positive bacteria, yeast, fungi, animal tissue, plant, etc.) can be homogenized in TRI Reagent[®] with a mortar/pestle, dounce, tissue grinder, or bead beating with a high-speed homogenizer² prior to RNA Purification (page 4).

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Biological Liquids

Add 3 volumes TRI Reagent® to each liquid sample, (3:1; see table below) and mix thoroughly for 5 minutes. To remove particulate debris, centrifuge and transfer the supernatant into an RNase-free tube. Proceed to RNA Purification (below).

| | |
|---|------------------|
| Blood, Plasma, Serum, Semen, CSF, Buffy coat, Body fluids, etc. | Add TRI Reagent® |
| 100 µl | 300 µl |

Reaction Clean-Up

For clean-up of enzymatic reactions, add 3 volumes TRI Reagent® to each liquid sample, (3:1; see table below) and mix thoroughly. Proceed to RNA Purification (below).

| | |
|---|------------------|
| <i>In vitro</i> transcription, DNase I digestion, Labeling reaction, etc. | Add TRI Reagent® |
| 100 µl | 300 µl |

(II) RNA Purification

All centrifugation steps should be performed at 10,000-16,000 x g for 30 seconds.

1. Add an equal volume ethanol (95-100%) to a sample lysed in TRI Reagent® or similar¹ and mix thoroughly.
2. Transfer the mixture into a **Zymo-Spin™ IIICG Column²** in a **Collection Tube** and centrifuge. Transfer the column into a **new** collection tube and discard the flow-through.

Recommended: **DNase I** treatment (in-column)³

(D1) Add 400 µl **RNA Wash Buffer** to the column and centrifuge.

(D2) In an RNase-free tube, add 5 µl **DNase I** (6 U/µl)*, 75 µl **DNA Digestion Buffer** and mix⁴. Add the mix directly to the column matrix.

(D3) Incubate at room temperature (20-30°C) for 15 minutes. Proceed to step 3.

3. Add 400 µl **Direct-zol™ RNA PreWash⁵** to the column and centrifuge. Discard the flow-through and repeat this step.
4. Add 700 µl **RNA Wash Buffer⁵** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube.
5. To elute RNA, add 100 µl of **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use ≥50 µl elution.

RNA⁶ can be used immediately or stored frozen at ≤-70°C.

Up to 100 µl of biological liquid per prep can be processed without having to reload the spin column.

Notes:

¹ TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

² To process samples >700 µl, reload the column and repeat Step 2 (or use a vacuum manifold, centrifuge the column and proceed with the protocol).

³ Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

⁴ Mix by gentle inversion.

* *Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A₂₆₀ units/ml of reaction mixture at 25°C.*

⁵ Before use, add ethanol to the buffer concentrate (Buffer Preparation, page 3).

⁶ For complete removal of PCR (RT) inhibitors from plant, soil and fecal samples, use the OneStep™ PCR Inhibitor Removal Kit, D6030.

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Appendices

RNA extraction from samples stored in DNA/RNA Shield™

Add 3 volumes TRI Reagent® to each sample homogenate in DNA/RNA Shield™ (3:1) and mix thoroughly. To remove particulate debris, centrifuge (12,000 x g for 1 minute) and transfer the supernatant into an RNase-free tube. Proceed to RNA Purification (page 4).

Notes:

¹ For detailed processing information, refer to the TRI-Reagent® product manual (or manufacturer's instructions for the reagent used).

² Alternatively, the aqueous phase can be processed with the RNA Clean & Concentrator kit, R1015.

³ Different cells may react differently to centrifugation forces and it is recommended to test the pelleting procedure with non-valuable samples first. Diluting RNA/later™ by 50% with cold PBS reduces solution density allowing for lower forces during cell pelleting.

RNA purification from aqueous phase after TRI Reagent® extraction

For samples that have already been phase separated in TRI Reagent® or similar¹, simply transfer the aqueous phase² containing RNA into an RNase-free tube. Add an equal volume ethanol (95-100%) to the aqueous phase (1:1) and mix thoroughly. Proceed to RNA Purification (page 4, step 2).

RNA extraction from samples stored in RNA/later™

Cells

Pellet cells³ at up to 5,000 x g and remove the RNA/later™ (supernatant) prior to RNA extraction. Then lyse the cell pellet in TRI Reagent® (Sample Preparation, page 3).

Note: To extract RNA from cells without reagent removal, use 10 volumes of TRI Reagent® per sample volume. Proceed to phase separation and process the aqueous phase. Simply transfer the aqueous phase containing RNA into an RNase-free tube. Then add an equal volume ethanol (95-100%) to the aqueous phase (1:1) and mix thoroughly. Proceed to RNA Purification (page 4, step 2).

Tissue

Remove tissue from RNA/later™ using forceps. Eliminate any excess reagent or crystals that may have formed and proceed immediately with extraction in TRI Reagent® (Sample Preparation, page 3).

Ordering Information

| Product Description | Catalog No. | Kit Size |
|--|-------------|------------|
| Direct-zol™ RNA MiniPrep Plus (TRI Reagent® <u>not</u> included) | R2070T | 10 preps. |
| | R2070 | 50 preps. |
| | R2072 | 200 preps. |
| Direct-zol™ RNA MiniPrep Plus (supplied with TRI Reagent®) | R2071 | 50 preps. |
| | R2073 | 200 preps. |

| Individual Kit Components | Catalog No. | Amount |
|--|-------------|--------|
| TRI Reagent® | R2050-1-50 | 50 ml |
| | R2050-1-200 | 200 ml |
| Direct-zol™ RNA PreWash (concentrate) | R2050-2-40 | 40 ml |
| | R2050-2-160 | 160 ml |
| RNA Wash Buffer (concentrate) | R1003-3-6 | 6 ml |
| | R1003-3-12 | 12 ml |
| | R1003-3-24 | 24 ml |
| | R1003-3-48 | 48 ml |
| Zymo-Spin™ IIICG Columns | C1006-50-G | 50 |
| Collection Tubes | C1001-50 | 50 |
| | C1001-500 | 500 |
| | C1001-1000 | 1000 |
| DNase/RNase-Free Water | W1001-1 | 1 ml |
| | W1001-4 | 4 ml |
| | W1001-6 | 6 ml |
| | W1001-10 | 10 ml |
| | W1001-30 | 30 ml |
| DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml) | E1010 | 1 set |

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