ZYMO RESEARCH

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INSTRUCTION MANUAL

Direct-zol™ RNA MicroPrep

Catalog Nos. **R2060, R2061, R2062,** & **R2063**

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

U.S. Patent No. 9,051,563 and other pending patents.

Ver. 1.0.0

ZYMO RESEARCH CORP.

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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.

Product Contents

Direct-zol™ RNA MicroPrep Kit Size (Preps)	**R2060** (50)	**R2061** (50)	**R2062** (200)	**R2063** (200)
TRI Reagent®	-	50 ml	-	200 ml
Direct-zol™ RNA PreWash[1] (concentrate)	40 ml	40 ml	160 ml	160 ml
RNA Wash Buffer[2] (concentrate)	12 ml	12 ml	48 ml	48 ml
DNase I[3] (lyophilized)	1	1	4	4
DNA Digestion Buffer	4 ml	4 ml	16 ml	16 ml
DNase/RNase-Free Water	4 ml	4 ml	10 ml	10 ml
Zymo-Spin™ IC Columns	50	50	200	200
Collection Tubes	100	100	400	400
Instruction Manual	1	1	1	1

Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.
TRI Reagent® is provided with catalog numbers **R2061** and **R2063** only.

[1] Before use, add 10 ml and 40 ml ethanol (95-100%) to the 40 ml and 160 ml **Direct-zol™ RNA PreWash** concentrate, respectively.
[2] 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.
[3] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial prior to use. Store frozen aliquots.

Specifications

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

- **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 R2061, R2063 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™ IC Column** is ~10 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 RNA*later*™ (page 5).
- **RNA Storage** – RNA eluted with **DNase/RNase-Free Water** (provided) can be stored at ≤-70 ºC. 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.

Product Description

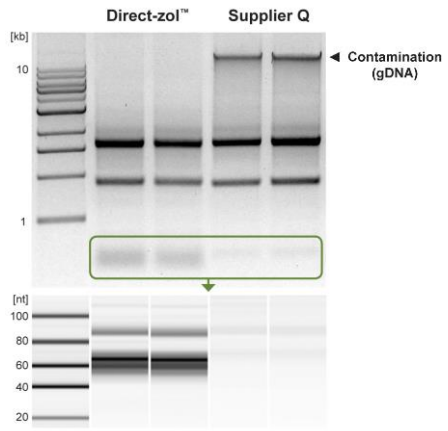
The **Direct-zol™ RNA MicroPrep** provides a streamlined method for the purification of up to 10 µg (per prep) of high-quality RNA *directly* from samples in TRI Reagent® or similar[1]. Total RNA, including small RNAs (17-200 nt), is effectively isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, biological liquids, *etc.*).

Isolation of RNA by conventional phase separation was shown[2] 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™ IC 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.

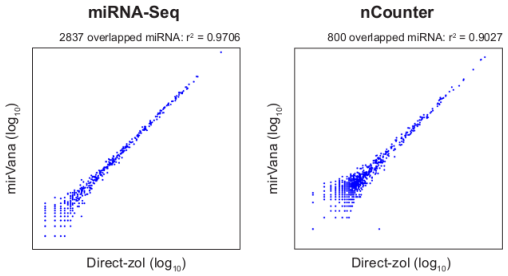
Efficient Recovery of Small & Large RNAs



(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

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

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

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 similar[1] *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).

Notes:

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

(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

* 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 cm^2 of culture surface area.

Tissue

Lyse tissue (≤ 5 mg) sample in 300 µl of TRI Reagent® and homogenize[2,3]. 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.

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 homogenizer[3] prior to RNA Purification (page 4).

[2] Squisher™ homogenizers, H1001, H1002, H1004.

[3] ZR BashingBead Lysis Tubes, S6002 and FastPrep®-24, S6005.

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

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

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).

In vitro 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 similar[1] and mix thoroughly.

2. Transfer the mixture into a **Zymo-Spin™ IC Column**[2] 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)*, 35 µl **DNA Digestion Buffer** and mix[4]. 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 PreWash**[5] to the column and centrifuge. Discard the flow-through and repeat this step.

4. Add 700 µl **RNA Wash Buffer**[5] 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 15 µl of **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated RNA use ≥6 µl elution.

   RNA[6] can be used immediately or stored frozen at ≤-70°C.

Notes:

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

[2] To process samples >700 ul, 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 A_{260} 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.

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).

RNA purification from aqueous phase after TRI Reagent® extraction

For samples that have already been phase separated in TRI Reagent® or similar[1], simply transfer the aqueous phase[2] 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 cells[3] 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).

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.

Ordering Information

Product Description	Catalog No.	Kit Size
Direct-zol™ RNA MicroPrep (TRI Reagent® not included)	R2060 R2062	50 preps. 200 preps.
Direct-zol™ RNA MicroPrep (supplied with TRI Reagent®)	R2061 R2063	50 preps. 200 preps.

Individual Kit Components	Catalog No.	Amount
TRI Reagent®	R2050-1-50 R2050-1-200	50 ml 200 ml
Direct-zol™ RNA PreWash (concentrate)	R2050-2-40 R2050-2-160	40 ml 160 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set

RNA MADE SIMPLE

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