ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Direct-zol™-96 RNA

Catalog Nos. **R2054, R2055, R2056 & R2057**

Highlights

- Quick, 96-well purification of high-quality (DNA-free) total RNA ***directly*** from TRIzol®, TRI Reagent® and all 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. 1.0.3

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™-96 RNA Kit Size (Preps)	**R2054** (2x 96)	**R2055*** (2x 96)	**R2056** (4x 96)	**R2057*** (4x 96)
TRI Reagent®	-	200 ml	-	2x 200 ml
Direct-zol™ RNA PreWash[1] (concentrate)	160 ml	160 ml	2x 160 ml	2x 160 ml
RNA Wash Buffer[2] (concentrate)	48 ml	48 ml	2x 48 ml	2x 48 ml
DNase I[3] (lyophilized)	4	4	8	8
DNA Digestion Buffer	2x 4 ml	2x 4 ml	16 ml	16 ml
DNase/RNase-Free Water	10 ml	10 ml	30 ml	30 ml
Zymo-Spin™ I-96 Plate	2	2	4	4
Collection Plate	4	4	8	8
Elution Plate	2	2	4	4
Cover Foil	2	2	4	4
Instruction Manual	1	1	1	1

Storage Temperature - Store all kit components (*i.e.,* buffers, plates) at room temperature.
*TRI Reagent® is provided only with catalog numbers R2055 & R2057.
[1] Before use, add 40 ml ethanol (95-100%) to the 160 ml **Direct-zol™ RNA PreWash** concentrate.
[2] Add 192 ml 100% ethanol (or 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, biological fluids (*e.g.,* blood, plasma, serum, CSF), and *in vitro* processed RNA (*e.g.,* transcription products, DNase-treated or labeled RNA)).
- **Sample inactivation** – TRI Reagent® (provided with R2055 & R2057) 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 performed with DNase I digestion (page 4).
- **RNA Recovery** – The RNA binding capacity of the **Zymo-Spin™ I-96 Plate** is ~10 µg/well.
- **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 (optional) is highly recommended for prolonged storage.
- **Equipment Needed** – Centrifuge with microplate carriers.

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.

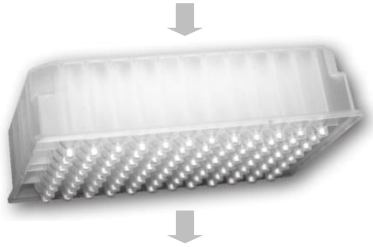
Product Description

The **Direct-zol™-96 RNA** provides a streamlined method for the purification of up to 10 µg (per well) of high-quality RNA *directly* from samples in TRI Reagent®[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.*) using this product. The extraction method inactivates viruses and other infectious agents.

The procedure is easy: simply apply a sample in TRI Reagent® to the **Zymo-Spin™ I-96 Plate**, then bind, wash, and elute the RNA. No phase separation, precipitation, or post-purification steps are necessary. The result is broad range purification of small and large RNAs suitable for subsequent RNA-based methods including RT-PCR, transcription profiling, hybridization, *etc*.

The entire procedure typically takes about 30 minutes (per 2 plates).

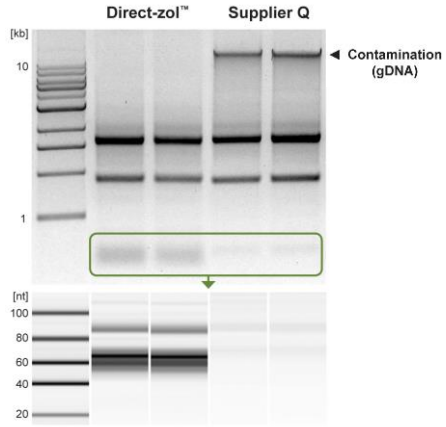
Sample in TRI Reagent®, TRIzol® or similar



Spin
Wash
Elute

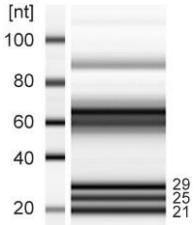
Ready-to-Use RNA

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).

Direct-zol™ Purification of miRNAs



Micro RNAs are effectively recovered from TRIzol® extracts using the **Direct-zol™** procedure. miRNAs (21-29 nt) "spiked" into the extract are evidenced by a Bioanalyzer (Small RNA Chip).

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.

Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

Notes:

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

Buffer Preparation

- ✓ Add 40 ml ethanol (95-100%) to 160 ml **Direct-zol™ RNA PreWash** concentrate.
- ✓ Add 192 ml 100% ethanol (or 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).

(I) Sample Preparation

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

1. To lyse a sample, resuspend cells or homogenize tissue in an appropriate volume (see table below) of TRI Reagent®, TRIzol® or similar[1] acid-guanidinium-phenol reagents.

Animal	Tissue	Biological Fluids	Add TRI Reagent®
≤ 10^6	≤ 5 mg	≤ 100 µl	300 µl

2. To remove particulate debris, centrifuge and transfer the supernatant into a **Collection Plate**.

(II) RNA Purification

All centrifugation steps should be performed at ≥ 2,500 x *g*.

1. Add an equal volume ethanol (95-100%) to a sample lysed in TRI Reagent® or similar[1] and mix well[2].

2. Transfer the mixture to a well of the **Zymo-Spin™ I-96 Plate**[3] mounted on a **Collection Plate** and centrifuge for 5 minutes. Mount the Zymo-Spin I-96 plate onto a **new** collection plate and discard the flow-through.

   Recommended: **DNase I** treatment (in-column)[4]

   (D1) Add 400 µl **RNA Wash Buffer** to each well of the plate and centrifuge.
   (D2) For each sample/well to be treated, mix 5 µl **DNase I** (6 U/µl)* and 35 µl **DNA Digestion Buffer** in an RNase-free tube and mix by inversion. Add 40 µl directly to the column matrix of each well.
   (D3) Incubate at room temperature (20-30°C) for 15 minutes. Proceed to step 3.

3. Add 400 µl/well **Direct-zol™ RNA PreWash**[5] to the plate and centrifuge for 5 minutes. Discard the flow-through and repeat this step.

4. Add 800 µl/well **RNA Wash Buffer**[5] to the plate and centrifuge for 5 minutes. Discard the flow-through. To ensure complete removal of the wash buffer, centrifuge the plate for an additional 5 minutes. Then mount the plate onto an **Elution Plate**.

5. Add 25 µl/well of **DNase/RNase-Free Water** directly to the matrix and centrifuge for 5 minutes.

   Alternatively, for highly concentrated RNA use ≥10 µl elution.

   The RNA can be used immediately or stored frozen.
   Use the **Cover Foil** to prevent evaporation.

All steps should be performed at room temperature unless specified otherwise.

Notes:

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

[2] **Mix well** by pipetting up and down several times and/or (if available) by vortexing at ~1,300 rpm.

[3] The well capacity is 800 µl. Reload the plate to process >800 µl.

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

* *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).

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™-96 RNA (TRI Reagent® is not included)	R2054 R2056	2 x 96 preps. 4 x 96 preps.
Direct-zol™-96 RNA (supplied with TRI Reagent®)	R2055 R2057	2 x 96 preps. 4 x 96 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™ I-96 Plates	C2004	2 plates
Collection Plates	C2002	2 plates
Elution Plates	C2003	2 plates
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