ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Direct-zol™ RNA MiniPrep

Catalog Nos. **R2050, R2051, R2052,** & **R2053**

Highlights

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

Contents

ZYMO RESEARCH CORP.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

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.

Follow applicable federal, state, and local regulations for phenol waste disposal.

This product is for research use only and not intended for use in diagnostic procedures.

Some technologies included in this product are patent pending.

Product Contents

Direct-zol™ RNA MiniPrep Kit Size (Preps)	**R2050** (50)	**R2051** (50)	**R2052** (200)	**R2053** (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 Set[3] DNase I (250 U) & 10X Reaction Buffer (1 ml)	1 set	1 set	4 sets	4 sets
DNase/RNase-Free Water	6 ml	6 ml	2x 6 ml	2x 6 ml
Zymo-Spin™ IIC 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 **R2051** and **R2053** 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] Reconstitute the lyophilized **Dnase I** prior to use and store at -20°C (Appendix A, page 6).

Specifications

- **Sample Sources** – Cells from culture, solid tissue, plasma, serum, whole blood, and *in vitro* processed RNA (*e.g.,* transcription products, DNase-treated or labeled RNA) or samples stored and preserved in TRI Reagent®, TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.
- **Sample Inactivation** – TRI Reagent® (provided with R2051, R2053 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 (Appendix A, page 6).
- **RNA Recovery** – The RNA binding capacity of the provided **Zymo-Spin™ IIC Column** is ~50 µg.
- **Compatibility** – TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* based solutions can be used in place of TRI Reagent®.

  Note: Compatible with samples stored in RNA*later*™ (Appendix B, page 6). Also, compatible with samples in TRI Reagent® that contain chloroform, 1-bromo-3-chloropropane (BCP), or 4-bromoanisole (BAN) *or* the aqueous phase of phase-separated samples (Appendix C, page 6).
- **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.

Product Description

Ready-to-Use RNA

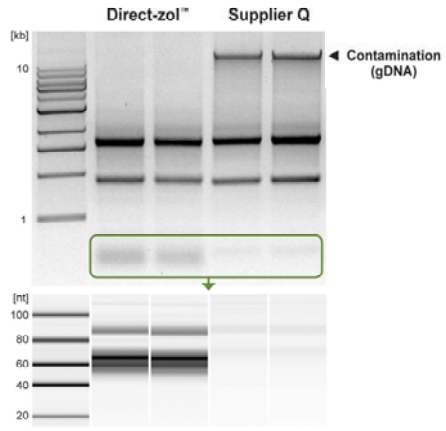
The **Direct-zol™ RNA MiniPrep** provides a streamlined method for the purification of up to 50 µg (per prep) of high-quality RNA *directly* from samples in TRI Reagent® or similar[1]. Total RNA, including small RNAs (17-200 nt), is effectively isolated from a variety of sample sources (cells, tissues, 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™ IIC 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 10 minutes.

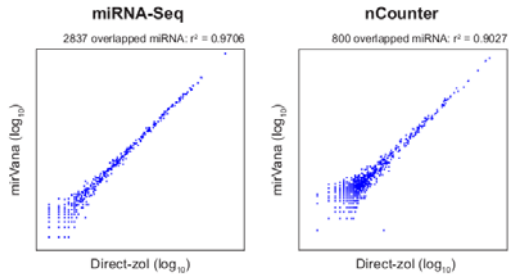
Efficient Recovery of Small & Large RNAs



(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 can be performed at room temperature.

Buffer Preparation

- ✓ Add 10 ml and 40 ml ethanol (95-100%) to the 40 ml and 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.

Sample Preparation

The following guidelines are provided for processing[1] various sample types in TRI Reagent® or similar[2] prior to spin column purification of the RNA.

Cell Monolayers

It is recommended to process between 5x10^3 - 5x10^6 animal cells (per prep).

1. Lyse adherent cells[3] directly in a culture plate/dish. Add 100 µl TRI Reagent® for each cm^2 of culture surface area and mix well by pipetting. Incubate the mixture for 5 minutes at room temperature.

   Example: Add 200 µl TRI Reagent® per well of a 24-well plate (table below).

2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Approximate cell number per culture area for "high-density" growth cells.

Culture Container	Well /Flask Surface Area	Cell Number
96-well plate	0.32-0.6 cm^2	4-5x10^4
24-well plate	2 cm^2	1-3x10^5
12-well plate	4 cm^2	4-5x10^5
6-well plate	9.5 cm^2	0.5-1x10^6
T25 Culture Flask	25 cm^2	2-3x10^6
T75 Culture Flask	75 cm^2	0.6-1x10^7
T175 Culture Flask	175 cm^2	2-3x10^7

Cell Suspensions

It is recommended to process between 5x10^3 - 5x10^6 animal cells (per prep)[4].

1. Pellet cells by centrifugation. Carefully remove the supernatant and lyse the cell pellet directly in TRI Reagent®. Use 1 ml of the TRI Reagent® for up to 10^7 animal cells.

   Note: Alternatively, for cell suspensions, add 3 volumes of TRI Reagent® to each volume of cell suspension.

   Mix well by vortexing and incubate the mixture for 5 minutes at room temperature.

2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Notes:

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

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

[3] The procedure is compatible with high-density growth cells (e.g., HeLa cells) as well as low-density ones (e.g., neuronal cells).

[4] For homogenization of tough-to-lyse microbial samples, use **ZR BashingBead™ Lysis Tubes** (S6002-50) with disrupters/pulverizers fitted with a 2 ml tube holder assembly.

Biological Liquids

Up to 100 µl of biological liquid per prep (*e.g.,* blood, serum, plasma, semen, CSF, buffy coat, body fluids) can be processed without having to reload the spin column.

1. Add three volumes of TRI Reagent® to each volume of liquid sample. Mix well by vortexing and incubate the mixture for 5 minutes at room temperature.
2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Tissue

An equivalent of up to 50 mg tissue (per prep) can be sampled with this kit. Larger samples can exceed the RNA binding capacity of the spin column.

1. Add at least 500 µl TRI Reagent® per 50 mg tissue. Homogenize using **ZR BashingBead™ Lysis Tubes**[1], **Squisher™**[2], a glass-Teflon, Polytron, or similar homogenizer.

   Note: Sample should not exceed 10% of the TRI Reagent® volume used for homogenization.
2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

In vitro Reactions

For cleanup of enzymatic reactions (*e.g., in vitro* transcription products, DNase-treated or labeled RNA), add three volumes TRI Reagent® to each volume of sample and mix by vortexing. Proceed with the **RNA Purification** (page 5).

Example: Add 300 µl TRI Reagent® to a 100 µl reaction.

Samples already homogenized in TRI Reagent®

Remove particulates from cell and tissue sample homogenates in TRI Reagent® by centrifugation at 12,000 *x g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Notes:

[1] For homogenization of tough-to-lyse small tissue samples, use **ZR BashingBead™ Lysis Tubes** (S6003-50) with disrupters/ pulverizers fitted with a 2 ml tube holder assembly.

[2] **Squisher™** homogenizers (H1001, H1002, H1004) are available from Zymo Research.

RNA Purification

All centrifugation steps should be performed between 10,000-16,000 x *g*.

1. Add one volume ethanol (95-100%) directly to one volume sample homogenate (1:1) in TRI Reagent® or similar[1]. Mix well by vortexing.

2. Load the mixture into a **Zymo-Spin™ IIC Column**[2] in a **Collection Tube** and centrifuge for 1 minute. Transfer the column into a new **Collection Tube** and discard the **Collection Tube** containing the flow-through.

   At this point, RNA samples can be *in-column* DNase I treated (Appendix A, page 6).

3. Add 400 µl **Direct-zol™ RNA PreWash**[3] to the column and centrifuge for 1 minute. Discard the flow-through. Repeat this step.

4. Add 700 µl **RNA Wash Buffer**[3] to the column and centrifuge for 1 minute. Discard the flow-through. To ensure complete removal of the wash buffer, centrifuge the column for an additional 2 minutes in an emptied **Collection Tube**. Transfer the column carefully into an RNase-free tube (not provided).

5. Add 50 µl of **DNase/RNase-Free Water**[4] directly to the column matrix and centrifuge for 1 minute.

   Alternatively, for highly concentrated RNA use ≥25 µl elution.

   The eluted RNA can be used immediately or stored 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.

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

[4] For maximum recovery, increase the elution volume (≥50 µl) and/or repeat the elution.

DNase/RNase-Free Water preheated to 95º C can also be used for elution.

Appendix A: In-Column DNase I digestion

All centrifugation steps should be performed between 10,000-16,000 x *g*.

1. Following the RNA binding step (page 5, step 2), wash the column with 400 µl **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.
2. For each sample to be treated, prepare **DNase I reaction mix** in an RNase-free tube (not provided). Add the reagents in the following order:

(1) **DNase I**[1]	5 µl (1 U/µl)
(2) **10X DNase I Reaction Buffer**	8 µl
(3) **DNase/RNase-Free Water**	3 µl
(4) **RNA Wash Buffer** (with ethanol added)	64 µl
	80 µl

3. Add 80 µl of the **DNase I Reaction Mix** directly to the column matrix. Incubate the column at room temperature (20-30 ºC) for 15 minutes[2], then centrifuge for 30 seconds.

   Continue with RNA Purification (page 5, step 3).

Appendix B: 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 immediately lyse the cell pellet in TRI Reagent®[4,5] (Sample Preparation, Cell Suspensions, 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 (not provided). Then, proceed to RNA Purification (page 5).

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® or similar[4] (Sample Preparation, Tissue, page 4).

Appendix C: Aqueous phase of phase-separated samples

For samples that have already been phase separated in TRI Reagent® or similar[5], simply transfer the aqueous phase containing RNA into an RNase-free tube (not provided). Then, proceed to RNA Purification (page 5).

Notes:

[1] To reconstitute the lyophilized **DNase I** (E1009) at 1 U/µl, add 275 µl **DNase/RNase-Free Water** (per vial). Mix by gentle inversion. Store aliquots at -20 ºC.

Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

[2] The optimal incubation time can vary.

[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.

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

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

Ordering Information

Product Description	Catalog No.	Kit Size
Direct-zol™ RNA MiniPrep (TRI Reagent® not included)	R2050 R2052	50 preps. 200 preps.
Direct-zol™ RNA MiniPrep (supplied with TRI Reagent®)	R2051 R2053	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™ IIC Columns	C1011-50 C1011-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
DNase/RNase-Free Water	W1001-1 W1001-6 W1001-10	1 ml 6 ml 10 ml
DNase I (lyophilized) (250 U supplied with 10X Reaction Buffer)	E1009	1 set