



ZYMO RESEARCH

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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 *TRI-Reagent*® or similar acid-guanidinium-phenol based reagents (TRIzol®, RNAzol®, QIAzol®, TriPure, RNA-Bee *etc.*).
- Bypasses phase separation and precipitation procedures.
- Efficient, broad range purification of small and large RNAs from cells, tissues, biological liquids, *in vitro* transcripts, *etc.*
- Ideal for viral inactivation/sample storage*.

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

Product Contents

Direct-zol™ RNA MiniPrep Kit Size (Preps.)	R2050 (50)	R2051 (50)	R2052 (200)	R2053 (200)
TRI-Reagent®	-	50 ml	-	100 ml
Direct-zol™ RNA PreWash¹ (concentrate)	40 ml	40 ml	160 ml	160 ml
RNA Wash Buffer² (concentrate)	12 ml	12 ml	48 ml	48 ml
DNase I Set³ DNase I (250 U) & 10x DNase I 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	2x 50	2x 50	8x 50	8x 50
Instruction Manual	1	1	1	1

Note - 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.

Storage Temperature - Store all kit components (*i.e.*, buffers, columns) at room temperature.

TRI-Reagent® (50 ml and 100 ml) is provided only with catalog numbers **R2051** and **R2053**, respectively.

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 Add 275 µl **DNase/RNase-Free Water** to reconstitute the lyophilized **DNase I** (250 U; ~1 U/µl after reconstitution).

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**, **RNA-Bee** and other *acid-guanidinium-phenol* reagents.
- **Sample inactivation** – **TRI-Reagent®** (provided only with R2051, R2053) 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 *in-column* DNase I digestion (*see Appendix A, page 6*).
- **RNA Recovery** – The RNA binding capacity of the supplied **Zymo-Spin™ IIC Column** is ~50 µg with a minimal elution volume of 25 µl.
- **Compatibility** – **TRIZol®**, **RNAzol®**, **QIAzol®**, **TriPure**, **RNA-Bee** or similar *acid-guanidinium-phenol* based solutions can be used in place of **TRI-Reagent®**.

Note: Compatible with samples stored in **RNAlater™** (*see 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.
- **RNA Storage** – RNA eluted with the **DNase/RNase-Free Water** (provided) can be stored at ≤-70 °C. The addition of RNase inhibitors (optional) is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

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

™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

TRI-Reagent®, **TRIZol®**, **RNAzol®** are registered trademarks of Molecular Research Center, Inc. **QIAzol®** is a registered trademark of Qiagen GmbH. **TriPure** is a trademark of Roche. **RNA-Bee** is a trademark of Tel-Test, Inc. **RNAlater™** is a trademark of Ambion, Inc., Austin, Texas and is protected by various U.S. and foreign patents.

Some technologies included in this product are patent pending.

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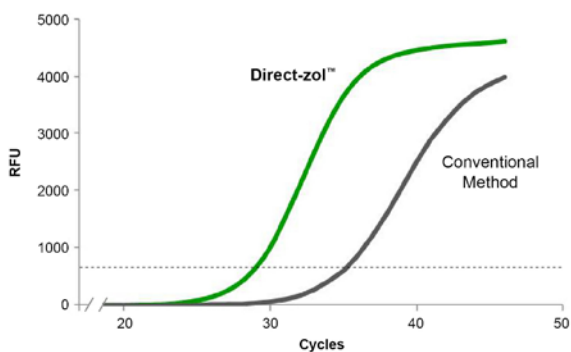
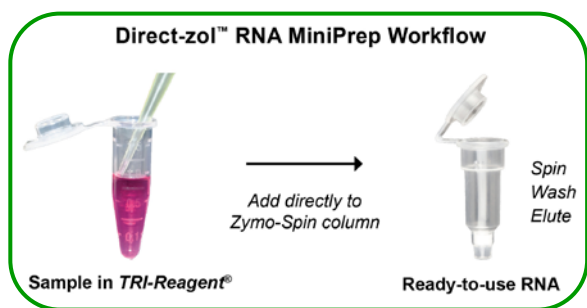
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Product Description

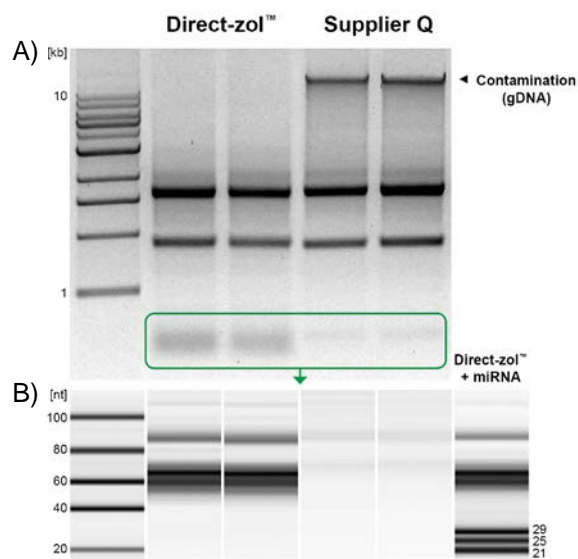
The **Direct-zol™ RNA MiniPrep** provides a streamlined method for the purification of up to 50 µg (*per prep*) of high-quality RNA *directly* from samples in *TRI-Reagent®* or other *acid-guanidinium-phenol* based reagents (*see Specifications*). The extraction method inactivates viruses and other infectious agents.* Total RNA including small and non-coding 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 procedure is easy: simply apply a sample in *TRI-Reagent®* to the spin column and then spin, 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 10 minutes.



Viral RNA is detected with high sensitivity following the Direct-zol™ isolation method. The Direct-zol™ method significantly improves the detection of West Nile virus when compared to the conventional phase-separation method. The RT-qPCR data show $\Delta C_t = 5$ (average of two independent experiments). RNA was isolated from cell-free samples inactivated using the *TRI-Reagent®*.



High quality broad range RNA is purified with the Direct-zol™ RNA MiniPrep. (A) *DNA-free* RNA purified from human epithelial cells using the Direct-zol™ RNA MiniPrep compared to a DNA containing preparation from Supplier Q (1% agarose/TAE). (B) Small RNAs are effectively recovered with the Direct-zol™ procedure while absent in Supplier Q preparations (Agilent Bioanalyzer 2100, Small RNA Chip data shown).

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

Note:

* For Catalog Nos. R2051, R2053 supplied with *TRI-Reagent®*.

Catalog Nos. R2050, R2052 do not include *TRI-Reagent®*.

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

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

Notes:

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

² Although cell types and culture conditions may vary, the procedure is compatible with high-density growth cells (e.g., HeLa cells) as well as low-density ones (e.g., neuronal cells).

³ For homogenization of tough-to-lyse microbial samples, use the **ZR BashingBead Lysis Tubes** (S6002-50) with disrupters/pulverizers fitted with a 2 ml tube holder assembly.

Buffer Preparation

- Add 10 ml and 40 ml ethanol (95-100%) to the 40 ml (R2050, R2051) and 160 ml (R2052, R2053) **Direct-zol™ RNA PreWash** concentrate, respectively.
- Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R2050, R2051) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R2052, R2053).

Sample Preparation Protocols

The following guidelines are provided for the processing¹ (homogenizing) various sample types in *TRI-Reagent*[®] or similar acid-guanidinium-phenol based reagents (*TRIZol*[®], *RNAzol*[®], *QIAzol*[®], *TriPure*, *RNA-Bee* etc.) prior to spin column **RNA Purification**.

Cell Monolayers

It is recommended to process 5×10^3 - 5×10^6 animal cells (*per prep*).

1. Lyse adherent cells² directly in a culture plate/dish. Add 100 μ l *TRI-Reagent*[®] for each cm² of culture surface area and mix well by pipetting. Incubate the mixture for 5 minutes at room temperature.

Example: Add 200 μ l *TRI-Reagent*[®] per well of a 24-well plate (*Table below*).

2. To remove particulate matter, centrifuge the mixture at 12,000 x g for 1 minute (or longer if necessary) and then carefully transfer the supernatant into a new tube. 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 5×10^3 - 5×10^6 animal cells (*per prep*).³

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 10⁷ animal cells.

Note: Alternatively, for dilute cell suspensions, add three 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 particulate matter, centrifuge the mixture at 12,000 x g for 1 minute (or longer if necessary) and then carefully transfer the supernatant into a new tube. Proceed with **RNA Purification** (*page 5*).

Biological Liquids

Up to 100 μ l 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.

Note: When sampling **whole blood** or **plasma** supplement each 100 μ l sample with 10 μ l of 5 N acetic acid, added prior to or after mixing the sample with *TRI-Reagent*[®].

2. To remove particulate matter, centrifuge the mixture at 12,000 x *g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into a new tube. 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 μ l *TRI-Reagent*[®] per 50 mg tissue. Homogenize using BashingBeads¹, a glass-Teflon, Polytron, Squisher[™] homogenizer² or similar.

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

2. To remove particulate matter, centrifuge the mixture at 12,000 x *g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into a new tube. 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 μ l *TRI-Reagent*[®] to a 100 μ l reaction.

Samples already homogenized in *TRI-Reagent*[®]

Remove particulate matter 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 a new tube. Proceed with **RNA Purification** (page 5).

Aqueous phase of phase-separated samples

Transfer the aqueous phase containing RNA into a new tube, then proceed with **RNA Purification** (page 5).

Notes:

¹ For homogenization of tough-to-lyse small tissue samples, use the **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 Protocol

The RNA binding capacity of Zymo-Spin IIC column is 50 µg.

1. Add 1 volume ethanol (95-100%) directly to the sample homogenate in *TRI-Reagent*[®] or similar (i.e., *TRIzol*[®], *RNAzol*[®], *QIAzol*[®], *TriPure*, *RNA-Bee* and other acid-guanidinium-phenol based reagents). Mix well by vortexing.

Alternatively: For phase-separated samples, add 1 volume ethanol (95-100%) to the aqueous phase.

Note: To process samples >700 µl, reload the column and repeat *Step 2* or use a vacuum manifold.

Notes:

¹ Before use, add ethanol to the buffer concentrate (see **Buffer Preparation**, page 3).

² For maximum recovery, increase the elution volume (≥50 µl) and/or repeat the elution.

2. Load the mixture into a **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge at ~12,000 x *g* for 1 minute. Transfer the column into a new **Collection Tube** and discard the **Collection Tube** containing the flow-through.

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

3. Add 400 µl **Direct-zol™ RNA PreWash**¹ to the column and centrifuge at ~12,000 x *g* for 1 minute. Discard the flow-through. **Repeat this step.**
4. Add 700 µl **RNA Wash Buffer**¹ to the column and centrifuge at ~12,000 x *g* for 1 minute.

Note: To ensure complete removal of the wash buffer, centrifuge the column at 12,000 x *g* for additional 2 minutes in an emptied Collection Tube.

Transfer the column carefully from the **Collection Tube** into an RNase-free tube (not provided).

5. Add ≥25 µl of **DNase/RNase-Free Water**² directly to the column matrix and centrifuge at max speed for 1 minute. The eluted RNA can be used immediately or stored at -70°C.

Appendix A: In-Column DNase I Digestion

The DNase digestion procedure can be performed using the **DNase I** with its **10X DNase I Reaction Buffer** (provided). Add 275 μl **DNase/RNase-Free Water** into each vial of **DNase I** (E1009) to reconstitute the enzyme. Mix by gentle inversion and store enzyme aliquots at -20°C . DNase I maintains activity in the **RNA Wash Buffer** provided with this product.

Make **DNase I cocktail** for each sample to be treated.

<i>Recommended:</i>	DNase I	5 μl (1 U/ μl)
	10X DNase I Reaction Buffer	8 μl
	DNase/RNase-Free Water	3 μl
	RNA Wash Buffer ¹	64 μl

1. Following *Step 2* in the protocol (*page 5*), add 400 μl **RNA Wash Buffer** to the **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge at $\sim 12,000 \times g$ for 1 minute. Discard the flow through.
2. Add the **DNase I cocktail** directly to the matrix of the **Zymo-Spin™ IIC Column**. Keep the column in the **Collection Tube**.
3. Incubate the column at $25\text{--}37^{\circ}\text{C}$ for 15 minutes², then centrifuge at $\sim 12,000 \times g$ for 30 seconds. Continue with *Step 3* in the protocol (*page 5*).

Notes:

¹ When adjusting volume and composition, make sure the **RNA Wash Buffer** in the **DNase I cocktail** remains at 80% (v/v).

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.

Appendix B: RNA extraction from samples stored in RNA/later™

Cells

Pellet cells³ at up to $5,000 \times g$ and remove the supernatant containing RNA/later™ prior to RNA extraction. Then immediately lyse the cell pellet in *TRI-Reagent*® (see **Sample Preparation, Cell Suspensions**, *page 3*).

Note: To extract RNA from cells in RNA/later™, use 10 volumes of *TRI-Reagent*® per sample volume. Proceed to phase separation and then process the aqueous phase (*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*® (see **Sample Preparation, Tissue**, *page 4*).

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

Ordering Information

Product Description	Catalog No.	Kit Size
Direct-zol™ RNA MiniPrep (<i>TRI-Reagent</i> ® not included)	R2050 R2052	50 preps. 200 preps.
Direct-zol™ RNA MiniPrep (supplied with <i>TRI-Reagent</i> ®)	R2051 R2053	50 preps. 200 preps.

Individual Kit Components	Catalog No.	Amount
<i>TRI-Reagent</i>®	R2050-1-50	50 ml
	R2050-1-100	100 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™ IIC Columns	C1011-50	50
	C1011-250	250
Collection Tubes	C1001-50	50
	C1001-500	500
	C1001-1000	1000
DNase/RNase-Free Water	W1001-1	1 ml
	W1001-6	6 ml
	W1001-10	10 ml
DNase I (lyophilized) (250 U supplied with 10x DNase I Reaction Buffer)	E1009	1 set

Related Products

Product	Description	Prep/Format	Catalog
Total RNA Purification From			
ZR Whole-Blood RNA MiniPrep™	whole blood, partitioned blood	50/column 100/column	R1020 R1021
ZR-96 Whole-Blood RNA Kit™		2x96/plate	R1022
ZR Viral RNA Kit™	plasma, serum, liquids, cells, tissue	50/column 200/column	R1034 R1035
ZR-96 Viral RNA Kit™		2x96/plate 4x96/plate	R1040 R1041
ZR Urine RNA Isolation Kit™	urine, liquid samples	50/column	R1039
Quick-RNA™ MicroPrep	cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids	50/column 200/column	R1050 R1051
Quick-RNA™ MiniPrep		50/column 200/column	R1054 R1055
Quick-RNA™ MidiPrep		25/column	R1056
ZR-96 Quick-RNA™		2x96/plate 4x96/plate	R1052 R1053
ZR RNA MicroPrep™	cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids; DNA removal column, small-RNA recovery (≥17nt), <i>in-column</i> DNase treatment protocol	50/column 200/column	R1060 R1061
ZR RNA MiniPrep™		50/column 200/column	R1064 R1065
Pinpoint™ Slide RNA Isolation System Kit I	fresh tissue sections	50/column	R1003
Pinpoint™ Slide RNA Isolation System Kit II	paraffin-embedded tissue	50/column	R1007
ZR Fungal/Bacterial RNA MicroPrep™	bacteria, yeast, fungi; BashingBead™ lysis	50/column	R2010
ZR Fungal/Bacterial RNA MiniPrep™		50/column	R2014
ZR Plant RNA MiniPrep™	plant parts and tissues; BashingBead™ lysis, RT/PCR inhibitor removal	50/column	R2024
ZR Tissue & Insect RNA MicroPrep™	insect, small tissue samples; BashingBead™ lysis	50/column	R2030
ZR Soil/Fecal RNA MicroPrep™	soil, sludge, sediment, feces	50/column	R2040
YeaStar RNA Kit™	yeast, fungi	50/column	R1002
RNA Clean-up, Concentration & Recovery From			
RNA Clean & Concentrator™-5	modified/labeled/impure/diluted RNA; small-RNA recovery (≥17nt); <i>acid phenol</i> extracted RNA directly from aqueous phase, <i>in-column</i> DNase treatment protocol	50/column 200/column	R1015 R1016
RNA Clean & Concentrator™-25		50/column 100/column	R1017 R1018
RNA Clean & Concentrator™-100		25/column	R1019
ZR-96 RNA Clean & Concentrator™		2x96/plate	R1080
DNA-Free RNA Kit™	DNase I treatment; small-RNA recovery (≥17nt)	50/column 200/column	R1013 R1014
Zymoclean™ Gel RNA Recovery Kit	agarose gel separated RNA	50/column	R1011
ZR small-RNA™ PAGE Recovery Kit	polyacrylamide gel separated RNA; small-RNA recovery (≥17nt)	20/column	R1070
DNA/RNA Parallel Purification From			
ZR-Duet™ DNA/RNA MiniPrep	cells, tissue, liquids; DNA/RNA separation, small-RNA recovery (≥17nt), <i>in-column</i> DNase treatment protocol	50/column	D7001
DNA/RNA Co-Purification From			
ZR Viral DNA/RNA Kit™	plasma, serum, liquids, cells, tissue	25/column 100/column	D7020 D7021

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