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*.

Contents

ZYMO RESEARCH CORP.

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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 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 DNase I Reaction Buffer (1 ml)	1 set	1 set	4 sets	4 sets
DNase/RNase-Free Water	4 ml	4 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 RNA*later*™ *(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 ºC. The addition of RNase inhibitors (optional) is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

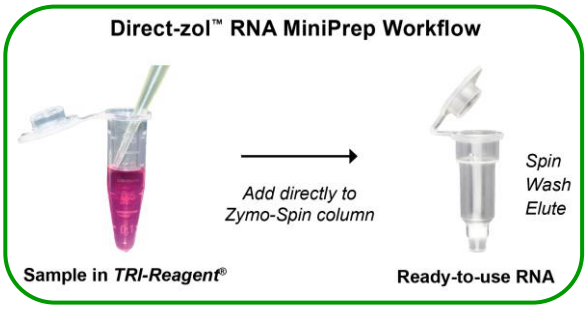
Follow applicable federal, state, and local regulations for phenol waste disposal.

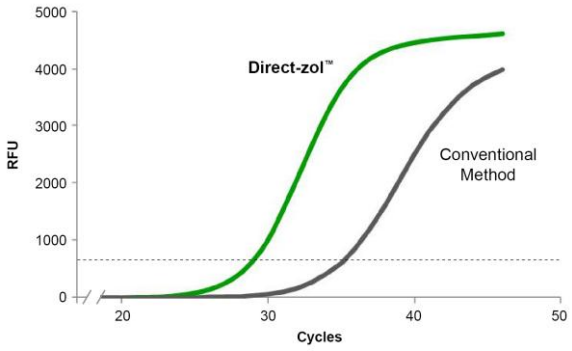
Product Description

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.

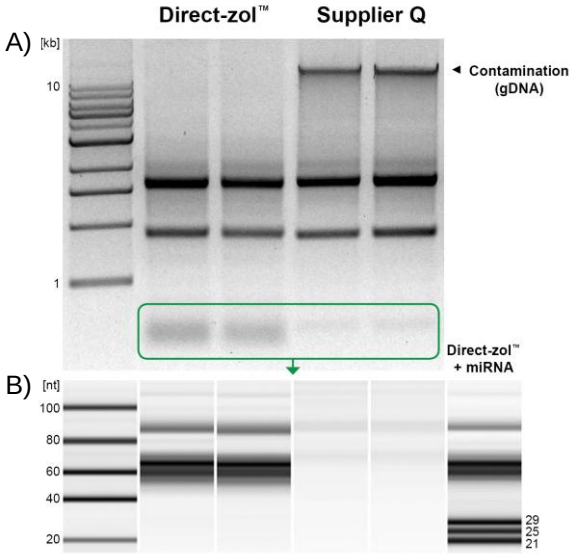
The procedure is easy: simply apply a sample in *TRI-Reagent®* to the spin column and then spin, wash, and elute the RNA. No phase separation, precipitation, or post-purification steps are necessary. The result is broad range purification of small and large RNAs suitable for subsequent RNA-based methods 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 ΔCt = 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.

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).

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

Sample Preparation Protocols

*The following guidelines are provided for the processing[1] (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 $5x10^3$ - $5x10^6$ animal cells *(per prep)*.

1. Lyse adherent cells[2] 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 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 $5x10^3$ - $5x10^6$ 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 10^7 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)*.

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.

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.

   Note: When sampling **whole blood** or **plasma** supplement each 100 µl sample with 10 µl 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 µl *TRI-Reagent*® per 50 mg tissue. Homogenize using BashingBeads[1], a glass-Teflon, Polytron, Squisher™ homogenizer[2] 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 µl *TRI-Reagent*® to a 100 µl 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.

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.

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.

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 -20ºC. DNase I maintains activity in the **RNA Wash Buffer** provided with this product.

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

Recommended:	**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 ~12,000 x *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 25-37ºC for 15 minutes[2], then centrifuge at ~12,000 x *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 cells[3] at up to 5,000 x *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 (*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 R2050-1-100	50 ml 100 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 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 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), *in-column* 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); *acid phenol* extracted RNA directly from aqueous phase, *in-column* 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), *in-column* 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**