ZYMO RESEARCH

RNA Purification Made Simple™

Direct-zol™ RNA Miniprep

TRIzol® In. RNA Out.

Highlights

- 7-minute, spin-column purification of total RNA (including small/microRNAs) ***directly*** from TRIzol®, TRI Reagent® or similar acid-guanidinium-phenol based reagents.
- No need for chloroform, phase-separation or precipitation steps.
- RNA is ready for Next-Gen Sequencing, RT-qPCR, etc. *DNase I is included.*

Catalog Numbers:
R2050, R2051, R2052, R2053

Scan with your smart-phone camera to view the online protocol/video.

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Table of Contents

Product Contents

Direct-zol™ RNA Miniprep	R2050 (50 prep)	R2051 (50 prep)	R2052 (200 prep)	R2053 (200 prep)
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)	1500 U	1500 U	1500 U (x4)	1500 U (x4)
DNA Digestion Buffer	4 ml	4 ml	16 ml	16 ml
DNase/RNase-Free Water	6 ml	6 ml	30 ml	30 ml
Zymo-Spin™ IICR Columns	50 pcs	50 pcs	200 pcs	200 pcs
Collection Tubes	100 pcs	100 pcs	400 pcs	400 pcs
Instruction Manual	1 pc	1 pc	1 pc	1 pc

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 10 ml or 40 ml ethanol (95-100%) to the 40 ml or 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.
3 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
#E1011-A (1500 U), add 275 µl **water**
#E1009-A (250 U), add 55 µl water

Specifications

- **Sample Sources –** Any sample stored and preserved in TRIzol®, TRI Reagent® or similar[1]. (animal cells, tissue, bacteria, yeast, biological fluids, samples stored in DNA/RNA Shield™ and in-vitro processed RNA (e.g., transcription products, DNase-treated or labeled RNA)).

- **Sample Inactivation –** TRI Reagent® (provided with **R2051**, **R2053** only) inhibits RNase activity and inactivates viruses and other infectious agents.

- **Size –** Total RNA including small/microRNAs (≥ 17 nt).

- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. RNA is ready for Next-Gen Sequencing, RT-qPCR, etc.

- **Binding Capacity –** 50 µg total RNA (**Zymo-Spin™ IICR Column**).

- **Compatibility –** TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol based reagents can be used in place of TRI Reagent®.

  Also, compatible with samples in TRIzol®, TRI Reagent® or similar reagent that contain chloroform, 1-bromo-3-chloropropane (BCP), or 4-bromoanisole (BAN), the aqueous phase of phase-separated samples and samples stored in RNAlater™ (page 7).

- **Elution Volume –** ≥ 25 µl **DNase/RNase-Free Water**.

- **Equipment**[2] **Needed** (user provided) **–** Microcentrifuge.

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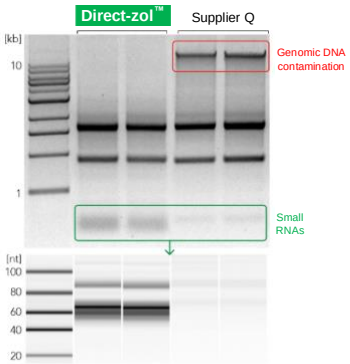
1 RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 For samples > 700 µl, the vacuum manifold can be used at step 2 only (page 6). Mount column onto the manifold and load sample. Then centrifuge the column to remove any residual buffer/sample. Proceed with the protocol by microcentrifuge.

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 TRIzol®, TRI Reagent® or similar[1]. Total RNA including small RNAs (17-200 nt)[2], is effectively isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, samples stored in DNA/RNA Shield™, etc.).

Simply add ethanol to a TRI Reagent® sample, bind directly to the **Zymo-Spin™ Column**, wash, and elute RNA. No phase separation, precipitation, or post-purification steps are necessary. RNA is high-quality and ready for Next-Gen Sequencing, RT-qPCR, transcription profiling, hybridization, etc.

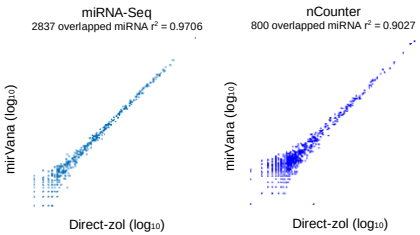
Efficient recovery of DNA-free total RNA



(top) High-quality 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).

Complete miRNA recovery



MicroRNA isolation using **Direct-zol™ RNA** kits**.** The data show RNA purified from TRIzol® samples using the Direct-zol™ RNA MiniPrep compared to a method known to be unbiased[2] (mirVana™, Ambion).
MicroRNA analysis was performed using miRNA-seq (MiSeq®, Illumina) and direct hybridization assay (nCounter®, Nanostring).

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1 RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 RNA isolation by conventional phase separation was shown to selectively enrich for some species of miRNA, leading to bias in downstream analysis (Kim et al. 2012. Molecular Cell 46(6):893-895). Direct-zol™ RNA method assures complete recovery of small/miRNAs.

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) RNA Purification.

The following guidelines are provided for processing various sample types in TRIzol®, TRI Reagent® or similar[1] acid-guanidinium-phenol reagents prior to column purification of the RNA (see page 8 for Input Capacity and Average RNA Yield).

(I) 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.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
  #E1011-A (1500 U), add 275 µl **water**
  #E1009-A (250 U), add 55 µl water

(II) Sample Preparation[2]

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 1 minute.

Cells

Lyse animal or gram(-) bacteria cells* directly in a culture dish** or resuspend pelleted cells in an appropriate volume (see table below) of TRI Reagent® or similar[1] and mix thoroughly. Proceed to RNA Purification (page 6).

Animal	Gram(-) bacteria	Add TRI Reagent®
≤ 10^5	-	≥ 100 µl
≤ 10^6	≤ 10^8	≥ 300 µl
≤ 5x10^6	≤ 5x10^8	≥ 600 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.

1 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 RNA yield can vary with sample types, organism, quality and treatment of the starting material (see page 8). To ensure complete lysis and homogenization of a sample, use a sufficient amount of TRIzol®, TRI Reagent® or similar reagent. For detailed processing information, refer to the TRI Reagent® product manual (or manufacturer's instructions for the reagent used).

Tough-to-lyse samples

Tough-to-lyse samples (see table below) can be homogenized in ≥ 800 µl TRIzol®, TRI Reagent® or similar[1] with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating with a high-speed homogenizer.

To remove particulate debris from homogenized tissue, centrifuge and transfer the supernatant into a new nuclease-free tube. Proceed to RNA Purification (page 6).

Recommended: Use ZR BashingBead™ Lysis Tubes (materials sold separately; #S6012, #S6003, #S014) for complete lysis and homogenization.

Input	Gram(-) bacteria (optional; easy-to-lyse)	Gram(+) bacteria	Tissue	Pathogen (microbes in tissue)
per prep	bacteria (≤ $5x10^8$)	bacteria (≤ $5x10^8$) yeast (≤ $5x10^7$)	animal: high yield (≤ 25 mg) animal: low yield (≤ 50 mg) plant (≤ 100 mg)	animal/insect, plant (≤ 25 mg)
lysis beads catalog #	0.5 mm and 0.1 mm; **S6012**	0.5 mm and 0.1 mm; **S6012**	2.0 mm; **S6003**	2.0 mm and 0.1 mm; **S6014**
high-speed[2,3]	30 sec	5-10 min	30-60 sec	3-5 min
low-speed[3]	5-10 min	20-40 min	3-5 min	5-10 min

Liquids

Add an appropriate volume of TRI Reagent® or similar[1] to a liquid sample and mix thoroughly (see table below). To remove particulate debris (if any), centrifuge and transfer the supernatant into an RNase-free tube. Proceed to RNA Purification (page 6).

Recommended: For biological samples (whole-blood, plasma, serum, buffy coat, PBMCs, WBCs, FACS, etc.) or samples collected in DNA/RNA Shield™[4], perform Proteinase K treatment[5] (sold separately), prior to adding TRI Reagent®.

Sample		Add TRI Reagent®
Biological liquid (blood, plasma, serum, WBCs, FACs, etc.) or **Reaction clean-up** (DNase I treated RNA, *in vitro* transcription, labeling, etc.).	100 µl	≥ 300 µl
Samples in DNA/RNA Shield™ (biological sample[4,5] or stored purified RNA).	100 µl	100 µl

1 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 Perform high-speed homogenization at 1-minute intervals (including a cooling step for 3-5 minutes), to avoid overheating the machine and/or breaking the tube.
3 High-speed homogenizers (e.g., MP Bio FastPrep-24™, Bertin Precellys, etc.). Low-speed homogenizers (e.g., Disruptor Genie, etc.).
4 DNA/RNA Shield™ reagent (R1100, R1200) or DNA/RNA Shield™ Blood Collection Tube (R1150).
5 For Proteinase K treatment, see page 9.

(III) RNA Purification

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

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™ IICR Column**[2] in a **Collection Tube** and centrifuge[3]. Transfer the column into a **new** collection tube and discard the flow-through.

3. **DNase I**[4] treatment (recommended)

   (D1) Add 400 µl **RNA Wash Buffer** to the column and centrifuge.

   (D2) In an RNase-free tube (not included), add 5 µl **DNase I** (6 U/µl)*, 75 µl **DNA Digestion Buffer** and mix by gentle inversion. Add the mix directly to the column matrix.

   (D3) Incubate at room temperature (20-30°C) for 15 minutes. Proceed to step 4.

4. Add 400 µl **Direct-zol™ RNA PreWash**[5] to the column and centrifuge. Discard the flow-through and repeat this step.

5. Add 700 µl **RNA Wash Buffer**[5] to the column and centrifuge for 1 minute to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not included).

6. To elute RNA, add 50 µl of **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

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

   The eluted RNA[6] can be used immediately or stored frozen.

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1 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 To process samples > 700 µl, reload the column and repeat Step 2 (or use a vacuum manifold, then centrifuge the column and proceed with the protocol).
3 At this point, proteins can be purified from the flow-through (see page 8).
4 Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 4). * 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 4).
6 For complete removal of PCR (RT) inhibitors from plant, soil and fecal samples, use the OneStep™ PCR Inhibitor Removal Kit (D6030).

Appendices

RNA purification from aqueous phase after TRI Reagent® extraction

For samples that have already been phase separated in TRI Reagent®[1] or similar[2], simply transfer the aqueous phase[3] 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 6, step 2).

RNA extraction from samples stored in RNAlater™

Cells

Pellet cells[4] at up to 5,000 x g and remove the RNAlater™ (supernatant) prior to RNA extraction. Then lyse the cell pellet in TRI Reagent® (Sample Preparation, Cells, page 4).

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 6, step 2).

Tissue

Remove tissue from RNAlater™ using forceps. Eliminate any excess reagent or crystals that may have formed and proceed immediately with extraction in TRI Reagent® (Sample Preparation, Tough-to-lyse samples, page 5).

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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™ or similar acid-guanidinium-phenol reagents.
3 Alternatively, the aqueous phase can be processed with the RNA Clean & Concentrator™ (R1015).
4 Different cells may react differently to centrifugation forces and it is recommended to test the pelleting procedure with non-valuable samples first. Diluting RNAlater™ by 50% with cold PBS reduces solution density allowing for lower forces during cell pelleting (e.g., 500 x g).

Protein Purification

The protein content in the flow-through after the RNA binding to the column can be purified (see RNA Purification, page 6, step 2):

1. Add 4 volumes of cold acetone (-20°C) to the flow-through (4:1) and mix.
2. Incubate the samples for 30 minutes on ice.
3. Centrifuge at max speed for 10 minutes. Discard the supernatant. Keep the pellet.
4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at max speed for 1 minute. Discard the supernatant.
5. Air-dry the protein pellet for 10 minutes at room temperature.
6. Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

Input Capacity and Average RNA Yield

Input	Average RNA Yield	Kit Capacity
Cells	10 µg (per 10^6 cells)	Up to $5x10^6$
HeLa	15 µg	
High Yield Tissue[1] (mouse)	≥ 30 µg (per 10 mg)	Up to 25 mg
Spleen	30-50 µg	
Liver	40-60 µg	
Low Yield Tissue[1] (mouse)	≤ 30 µg (per 10 mg)	Up to 50 mg
Brain, Heart	5-15 µg	
Muscle	5-20 µg	
Lung	10-20 µg	
Intestine	10-30 µg	
Kidney	20-30 µg	
Whole Blood[2]	(per 1 ml)	Up to 1 ml
Porcine	10-20 µg	
Human	2-10 µg	

Proteinase K Treatment

✓ Proteinase K treatment can be performed on protein-rich samples stored in **DNA/RNA Shield™** (2X concentrate; #R1200) (e.g., tissue, blood cells, plasma, serum, saliva, sputum, etc.) using **Proteinase K Set** (#D3001-2-5, D3001-2-20; sold separately).

Add 10 µl Proteinase K (reconstituted) to 1 ml DNA/RNA Shield sample (scale proportionally) and mix by inversion. Then incubate at room temperature (20-30°C) for 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.

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1 Yield from tissue can vary due to other factors (i.e., organism type, physiological state and growth conditions.
2 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

Ordering Information

Product Description	Catalog No.	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.

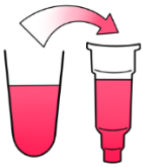
Individual Kit Components	Catalog No.	Amount
TRI Reagent®	R2050-1-50 R2050-1-200	50 ml 200 ml
Direct-zol™ RNA PreWash (concentrate)	R2050-2-40 R2050-2-160	40 ml 160 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
Zymo-Spin™ IICR Columns	C1078-50	50
Collection 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
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
Proteinase K Set (w/ Storage Buffer)	D3001-2-5 D3001-2-20	5 mg 20 mg

Complete Your Workflow

✓ For tough-to-lyse samples in TRIzol, use ZR BashingBead Lysis Tubes:

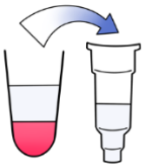
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ The only **direct**, high-throughput and automatable RNA purification from sample lysates in TRIzol (DNase I Set included with all formats):



Direct-zol RNA kits	
Microprep #R2060-R2063	From 1 cell and up
Miniprep #R2050-R2053	Up to 50 ug RNA
Miniprep Plus #R2070-R2073	Up to 100 ug RNA
96-well #R2054-R2057	Spin-plate
MagBeads #R2100-R2105	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

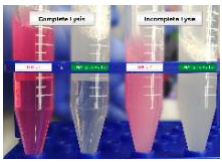


RNA Clean & Concentrator kit	
#R1013-R1014	DNase I Set included

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input: - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of TRIzol®, TRI Reagent® or similar reagent to ensure complete lysis and homogenization until lysate is transparent (see image). 
Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	Sample handling: - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. Incomplete lysis and/or cellular debris: - Increase the volume of TRIzol®, TRI Reagent® or similar to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
Low yield	Sample input: - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised RNA recovery. Use less input material and/or increase TRIzol®, TRI Reagent® or similar reagent. High-protein content (blood, plasma/serum, etc.) - Perform Proteinase K treatment to the sample prior to adding TRIzol®, TRI Reagent® or similar reagent (Sample preparation, Liquids, page 5).
DNA contamination	To remove DNA: - Perform in-column DNase I treatment (page 6) or perform DNase I treatment post-purification (R1013, page 4), then re-purify the treated sample. - For future preps, increase the volume of TRIzol®, TRI Reagent® or similar reagent to ensure complete lysis and homogenization of the sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into TRIzol®, TRI Reagent® or similar reagent to ensure RNA stability. Homogenized samples in TRIzol®, TRI Reagent® or similar can be stored frozen for later processing.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes

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Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not 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.

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