



Direct-zol[™] RNA Miniprep Plus

TRIzol[®] In. RNA Out.

Highlights

- spin-column purification of total 7-minute. 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: R2070T, R2070, R2071, R2072, R2073



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







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Product Contents

Direct-zol™ RNA Miniprep Plus	R2070T (50 prep)	R2070 (50 prep)	R2071 (50 prep)	R2072 (200 prep)	R2073 (200 prep)
TRI Reagent®	-	-	50 ml	-	200 ml
Direct-zol [™] RNA PreWash ¹ (concentrate)	10 ml	40 ml	40 ml	160 ml	160 ml
RNA Wash Buffer ²	16 ml (ready-to- use)	12 ml (concentrate)	12 ml (concentrate)	48 ml (concentrate)	48 ml (concentrate)
DNase I ³ (lyophilized)	250 U	1500 U	1500 U	1500 U (x4)	1500 U (x4)
DNA Digestion Buffer	0.8 ml	4 ml	4 ml	16 ml	16 ml
DNase/RNase-Free Water	1 ml	6 ml	6 ml	30 ml	30 ml
Zymo-Spin [™] IIICG Columns	10 pcs	50 pcs	50 pcs	200 pcs	200 pcs
Collection Tubes	20 pcs	100 pcs	100 pcs	400 pcs	400 pcs
Instruction Manual	1 pc	1 pc	1 pc	1 pc	1 pc

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

Before use:

#E1011-A (1500 U), add 275 µl **water** #E1009-A (250 U), add 55 µl water

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. RNA Wash Buffer included with the **R2070T** is supplied ready-to-use and does not require the addition of ethanol.

³ Reconstitute lyophilized DNase I with DNase/RNase-Free Water, mix by gentle inversion and store frozen aliquots:

Specifications

- Sample Sources Any sample stored and preserved in TRIzol[®], TRI Reagent[®] or similar¹. (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 R2071, R2073 only) inhibits RNase activity and inactivates viruses and other infectious agents.
- Size Total RNA including small/microRNAs (≥ 17 nt).
- **Purity** A₂₆₀/A₂₈₀ & A₂₆₀/A₂₃₀ > 1.8. RNA is ready for Next-Gen Sequencing, RT-qPCR, etc.
- Binding Capacity 100 µg total RNA (Zymo-Spin[™] IIICG 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 8).

- Elution Volume $\geq 50 \ \mu l$ DNase/RNase-Free Water.
- Equipment² Needed (user provided) Microcentrifuge.

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 7). Mount column onto the manifold and load sample. Then centrifuge the column to remove any residual buffer/sample. Proceed with the protocol by microcentrifuge.

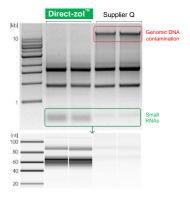
Product Description

The **Direct-zol**[™] **RNA Miniprep Plus** provides a streamlined method for the purification of up to 100 µg (per prep) of high-quality RNA directly from samples in TRIzol[®], TRI Reagent[®] or similar¹. 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.

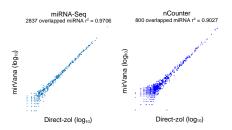
Efficient recovery of DNA-free Total RNA



(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 unbiased² (mirVana[™], Ambion).

MicroRNA analysis was performed using miRNAseq (MiSeq[®], Illumina) and direct hybridization assay (nCounter[®], Nanostring).

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.

Input Capacity and Average RNA Yield

Input	Average RNA Yield	Kit Capacity
Cells	10 µg (per 10 ⁶ cells)	Up to 10 ⁷
HeLa	15 µg	
High Yield Tissue ^{1 (mouse)}	≥ 30 µg (per 10 mg)	Up to 20 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 3 ml
Porcine	10-20 µg	
Human	2-10 µg	

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.

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 similar¹ acid-guanidinium-phenol reagents prior to column purification of the RNA (see page 4 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. RNA Wash Buffer included with the R2070T is supplied ready-to-use and does not require the addition of ethanol.
- ✓ 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 Preparation²

✓ 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 similar¹ 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
≤ 10 ⁷	≤ 10 ⁹	≥ 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 μ l for each cm² 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 4). 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 $\ge 800 \ \mu I$ TRIzol[®], TRI Reagent[®] or similar¹ 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 7).

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

Input	Gram(-) bacteria (optional; easy-to-lyse)	Gram(+) bacteria	Tissue	Pathogen (microbes in tissue)
per prep	bacteria (≤ 10 ⁹)	bacteria (≤ 10 ⁹) yeast (≤ 10 ⁸)	animal: high yield (≤ 20 mg) animal: low yield (≤ 50 mg) plant (≤ 200 mg)	animal/insect, plant (≤ 50 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 similar¹ 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 7).

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 treatment⁵ (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, <i>in vitro</i> transcription, 100 μI labeling, etc.).	≥ 300 µl
Samples in DNA/RNA Shield [™] (biological sample ^{4,5} or stored 100 μl purified RNA).	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 similar¹ and mix thoroughly.
- Transfer the mixture into a Zymo-Spin[™] IIICG Column² in a Collection Tube and centrifuge³. 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.
- Add 400 µl Direct-zol[™] RNA PreWash⁵ 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).
- To elute RNA, add 100 µl of DNase/RNase-Free Water directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use \geq 50 µl elution.

The eluted RNA⁶ can be used immediately or stored frozen.

¹ TRIzol[®], RNAzol[®], QIAzol[®], TriPure[™], TriSure[™] or similar acid-guanidinium-phenol reagent.

² To process samples > 700 μ l, 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 9).

⁴ Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 5). * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A₂₆₀ units/ml of reaction mixture at 25°C.

⁵ Before use, add ethanol to the buffer concentrate (Buffer Preparation, page 5).

⁶ For complete removal of PCR (RT) inhibitors from plant, soil and fecal samples, use the OneStep[™] PCR Inhibitor Removal Kit (D6030).

Appendices

<u>RNA purification from aqueous phase after TRI Reagent®</u> <u>extraction</u>

For samples that have already been phase separated in TRI Reagent^{®1} or similar², simply transfer the aqueous phase³ 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 7, step 2).

RNA extraction from samples stored in RNAlater[™]

Cells

Pellet cells⁴ 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 5).

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 7, 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 6).

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 7, 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.
- Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

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 μ I 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.

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 Plus (TRI Reagent [®] <u>not</u> included)	R2070T R2070 R2072	10 preps. 50 preps. 200 preps.
Direct-zol [™] RNA Miniprep Plus (supplied with TRI Reagent [®])	R2071 R2073	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 [™] IIICG Columns	C1006-50-G	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):

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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 6).
DNA contamination	To remove DNA:
	 Perform in-column DNase I treatment (page 7) or perform DNase I treatment post-purification (<u>R1013, page 4</u>), 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



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