ZYMO RESEARCH

RNA Purification Made Simple™

Direct-zol™ DNA/RNA Miniprep

TRIzol® In. RNA & DNA Out.

Highlights

- Spin-column purification of total RNA and DNA ***directly*** from TRIzol®, TRI Reagent® or similar acid-guanidinium-phenol based reagents.
- No need for chloroform, phase-separation or precipitation steps.
- DNA and RNA (including small/miRNAs) is ready for Next-Gen Sequencing, RT/qPCR, etc.

Catalog Numbers:
R2080T, R2080, R2081

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

Direct-zol™ DNA/RNA Miniprep	R2080T (10 prep)	R2080 (50 prep)	R2081 (50 prep)
TRI Reagent®	-		50 ml
RNA Prep Buffer	5 ml (x2)	25 ml (x2)	25 ml (x2)
RNA Wash Buffer[1]	16 ml (ready-to-use)	12 ml (concentrate)	12 ml (concentrate)
DNase/RNase-Free Water	1 ml	4 ml	4 ml
Direct-zol™ DNA Wash 1[2]	8 ml (ready-to-use)	24 ml (concentrate)	24 ml (concentrate)
Direct-zol™ DNA Wash 2	10 ml	40 ml	40 ml
Direct-zol™ DNA Elution Buffer	1 ml	4 ml	4 ml
Zymo-Spin™ IICR Columns	10 pcs	50 pcs	50 pcs
Collection Tubes	30 pcs	150 pcs	150 pcs
Instruction Manual	1 pc	1 pc	1 pc
DNA/RNA Shield™ Lysis Tube (Microbe)	Sold separately; #R1103 (50 pack)		
DNA/RNA Shield™ Lysis Tube (Tissue)	Sold separately; #R1105 (50 pack)		

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate. **RNA Wash Buffer** supplied in **R2080T** is ready-to-use (ethanol already added).
2 Add 15.5 ml 100% ethanol (16 ml 95% ethanol) to the 24 ml **Direct-zol™ DNA Wash 1** concentrate. **Direct-zol™ DNA Wash 1** supplied in **R2080T** is ready-to-use (ethanol already added).

Specifications

- **Sample Sources –** Cells (animal, bacterial, yeast), tissue (animal, plant), or biological liquids (blood, plasma, serum, CSF, buffy coat, etc.), freshly lysed in TRIzol®, TRI Reagent® or similar acid-guanidinium-phenol based reagent[1].

- **Sample Inactivation –** TRI Reagent® (provided with **R2081** only) inhibits nuclease activity and inactivates viruses and other infectious agents.

- **Size –** 50 bp to 10 kb (DNA), ≥ 17 nt (RNA).

- **Purity –** DNA and RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

- **Binding Capacity –** 25 µg DNA and 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 8)

- **Elution Volume –** ≥ 25 µl **DNase/RNase-Free Water** or **Direct-zol™ DNA Elution Buffer**.

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

Did you know?

TRIzol®/TRI Reagent® and similar[1] reagents are optimized for RNA isolation (low pH). DNA yield will vary with type and treatment of the sample. For optimal DNA isolation, use samples freshly lysed in TRIzol®/TRI Reagent® (pages 5-6). Freeze sample lysate (-80°C) for later processing.

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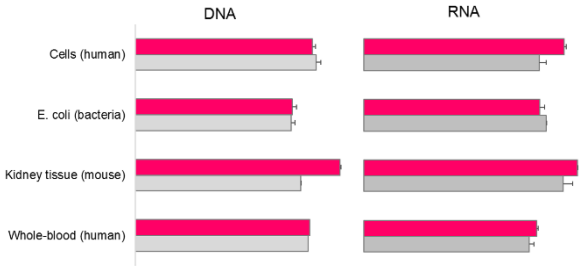
1 RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 For samples > 700 µl, the vacuum manifold can be used at step 2 only (page 6). Mount column onto the manifold and load sample. Then centrifuge the column to remove any residual buffer/sample. Proceed with the protocol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

Product Description

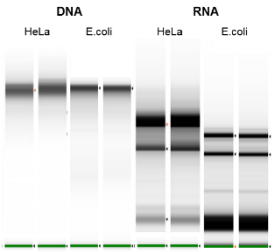
The **Direct-zol™ DNA/RNA Miniprep** kit provide an innovative method for the purification of DNA and total RNA from a variety of samples freshly lysed in TRIzol® or similar[1], including animal cells, tissue, bacteria, yeast, plant, biological liquids and etc.

Upon lysis of the sample with TRIzol® or similar[1], RNA and DNA is bound directly to the **Zymo-Spin™ Column**. Then simply spin, wash, and elute high-quality RNA and DNA into separate fractions. No phase separation, precipitation, or post-purification steps are necessary. The eluted nucleic acids are suitable for all subsequent molecular manipulations and analyses including Next-Gen sequencing, RT/qPCR, hybridization, etc.

Direct-zol™ DNA/RNA



Direct-zol™ DNA/RNA kit (pink bars) efficiently recovers both DNA and RNA from a single sample input as compared to DNA and RNA isolated with the Quick-DNA™ and Quick-RNA™ kits (light and dark gray bars, respectively). Data represent RT/qPCR C_T values.



High-quality DNA and RNA purified from mammalian and bacterial cells using the **Direct-zol™ DNA/RNA** kit (Agilent 2200 TapeStation).

One input. One column.

Any sample in TRIzol®
(TRI Reagent, etc.)

↓

Bind
(Zymo-Spin™ Column)

↓

Wash
Elute **RNA**

↓

Wash
Elute **DNA**

RNA & DNA
in separate fractions

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

Input Capacity and Average RNA Yield

Input	Average gDNA Yield	Average RNA Yield	Kit Capacity
Cells	4 µg (per 10^6 cells)	10 µg (per 10^6 cells)	Up to $5x10^6$
HeLa	6 µg	15 µg	
High Yield Tissue[1] (mouse)	≥ 30 µg (per 10 mg)	≥ 30 µg (per 10 mg)	Up to 10 mg
Spleen	50-70 µg	30-50 µg	
Liver	15-30 µg	40-60 µg	
Low Yield Tissue[1] (mouse)	≤ 30 µg (per 10 mg)	≤ 30 µg (per 10 mg)	Up to 25 mg
Brain, Heart	5-15 µg	5-15 µg	
Muscle	5-15 µg	5-20 µg	
Lung	15-30 µg	10-20 µg	
Intestine	15-30 µg	10-30 µg	
Kidney	15-30 µg	20-30 µg	
Whole Blood[2]	(per 1 ml)	(per 1 ml)	Up to 1 ml
Porcine	5-10 µg	10-20 µg	
Human	2-5 µg	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, (III) RNA Purification and (IV) DNA 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 and DNA. Nucleic acid yield can vary with sample type, organism, quality and treatment of the starting material (i.e., lysis and incubation time in TRIzol® or similar[1]).

(I) Buffer Preparation

- ✓ 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.
- ✓ Add 15.5 ml 100% ethanol (16 ml 95% ethanol) to the 24 ml **Direct-zol™ DNA Wash 1** concentrate. **Direct-zol DNA Wash 1** supplied in R2080T is ready-to-use (ethanol already added).

(II) Sample Preparation

- ✓ 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 of TRI Reagent® or similar[1] (see table below) and mix thoroughly. Incubate the lysate at room temperature for 1 hour. Centrifuge to remove debris (if any) and transfer the cleared supernatant into a nuclease-free tube (not provided). Proceed to RNA Purification (page 7).

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.

Tough-to-lyse samples

Add tough-to-lyse sample (see table below) to ≥ 800 µl TRI Reagent® or similar[1] in a nuclease-free tube (not included). Gently homogenize (i.e., dounce, mortar/pestle, syringe, etc.) and incubate the lysate at room temperature for 1 hour. Then centrifuge debris and process the cleared lysate. Proceed to RNA Purification (page 7).

Optional: For maximum yield, mechanical homogenization using high efficiency bead beating (i.e., DNA/RNA Shield™ Lysis Tube, Microbe #R1103 and/or Tissue #R1105 – materials sold separately) and a high-speed homogenizer can be used.

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 (≤ 10 mg) animal: low yield (≤ 25 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). Incubate the lysate at room temperature for 1 hour. Centrifuge to remove particulate debris (if any) and process the cleared lysate. 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 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**[6] (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.
6 **Reaction clean-up** must be performed with the RNA Purification protocol only, page 7.

(III) RNA Purification

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ **IMPORTANT:** RNA Purification must be performed prior to DNA Purification.

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. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through and repeat this step.

4. Add 700 µl **RNA Wash Buffer**[4] to the column and centrifuge for 1 minute to ensure complete removal of the wash buffer. Transfer the column carefully into a nuclease-free tube (not included).

5. To elute RNA[5], add 50 µl of **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   The eluted RNA can be used immediately or stored frozen.

(IV) DNA Purification

6. Add 700 µl **Direct-zol™ DNA Wash 1**[4] to the column and centrifuge. Discard the flow-through.

   For whole-blood samples (follow step 6 above), centrifuge the column again and then carefully transfer it into a nuclease-free tube (not included). Proceed to step 8 below.

7. Add 700 µl **Direct-zol™ DNA Wash 2** to the column and centrifuge for 1 minute to ensure complete removal of the wash buffer. Carefully, transfer the column into a nuclease-free tube (not included).

8. To elute DNA[5], add 50 µl of **Direct-zol™ DNA Elution Buffer** directly to the column matrix and centrifuge.

   The eluted DNA 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 9).
4 Before use, add ethanol to the buffer concentrate (Buffer Preparation, page 5).
4 For high concentrated RNA and/or DNA, use ≥ 25 µl elution volume.

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 tissue 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 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).

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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 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.
6. 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 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.

Ordering Information

Product Description	Catalog No.	Size
Direct-zol™ DNA/RNA Miniprep (TRI Reagent® not included)	R2080T R2080	10 preps. 50 preps.
Direct-zol™ DNA/RNA Miniprep (supplied with TRI Reagent®)	R2081	50 preps.

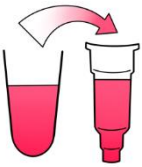
Individual Kit Components	Catalog No.	Amount
TRI Reagent®	R2050-1-50 R2050-1-200	50 ml 200 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-12 R1003-3-24	12 ml 24 ml
Zymo-Spin™ IICR Columns	C1078-50	50
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
Direct-zol DNA Wash 1 (concentrate)	R2080-1-24	24 ml
Direct-zol DNA Wash 2	R2080-2-40	40 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6	1 ml 4 ml 6 ml
Direct-zol™ DNA Elution Buffer	R2080-3-4	4 ml
DNA/RNA Shield™ Lysis Tube (Microbe)	R1103	50 tubes
DNA/RNA Shield™ Lysis Tube (Tissue)	R1105	50 tubes

Complete Your Workflow

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

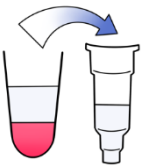
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ 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):

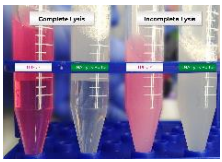


RNA Clean & Concentrator kit	
#R1013-R1014	DNase I Set included

✓ 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). 
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.
DNA yield and/or purity	For maximum DNA yield/purity, lyse a fresh sample in TRIzol®, TRI Reagent® or similar reagent and proceed with the protocol, Sample Preparation, pages 5-6. TRIzol®, TRI Reagent® and similar reagents are optimized for RNA isolation (low pH). DNA yield will vary with type and treatment of the sample.
DNA detection and quantification	DNA isolated from TRIzol®, TRI Reagent® or similar reagent will be detectable/usable via qPCR, NGS or etc. Eluted DNA is ready for any downstream application (for example, see data, page 3). For quantification with Qubit, NanoDrop™ and Bioanalyzer/TapeStation, use RNA parameters and/or RNA reagents.
DNA contamination	To remove DNA: - Perform DNase I treatment post-RNA 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.

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

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100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, 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.

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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