ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Direct-zol™ DNA/RNA Miniprep

Catalog Nos. **R2080T, R2080 & R2081**

Highlights

- **One Input, One Column:** Spin-column purification of RNA and DNA *directly* from samples in TRIzol®, TRI Reagent® and similar acid-guanidinium-phenol based reagents.
- **Easy Handling:** Bypass chloroform, phase separation and precipitation steps.
- **High-Quality:** DNA, RNA (including small/miRNAs) is ready for Next-Gen Sequencing, RT/PCR, hybridization, etc.

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ZYMO RESEARCH CORP.

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

Product Contents:

Direct-zol™ DNA/RNA Miniprep (Kit Size)	R2080T (10 Preps)	R2080 (50 Preps)	R2081 (50 Preps)
TRI Reagent®	-	-	50 ml
RNA Prep Buffer	2x 5 ml	2x 25 ml	2x 25 ml
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™ IIC Columns	10	50	50
Collection Tubes	30	150	150
Instruction Manual	1	1	1
DNA/RNA Shield™ Lysis Tube (Microbe)	Sold separately; #R1103 (50 pack)		
DNA/RNA Shield™ Lysis Tube (Tissue)	Sold separately; #R1105 (50 pack)		

Note - Integrity of kit components are guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

Store all kit components (reagents and plastics) at room temperature (20-25°C), unless specified.

[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 Type:** 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[1].
- **Purity:** DNA and RNA ready for all subsequent downstream analyses (Next-Gen sequencing, RT/qPCR)
- **Binding Capacity:** 25 µg DNA and 50 µg RNA (yield will vary with type and treatment of sample)
- **Size:** up to and above 40 kb (DNA), ≥17 nt (RNA)
- **Elution Volume:** ≥25 µl
- **Required Equipment:** Microcentrifuge
- **Sample Inactivation:** TRI Reagent® (provided with R2081 only) inactivates viruses and other infectious agents.
- **Compatibility:** Aqueous (RNA) phase from TRIzol®, TRI Reagent® or similar[1] can be processed. Also, compatible with samples in DNA/RNA Shield™ (see Appendix, page 6).

Did you know?

TRIzol®/TRI Reagent® and similar[1] reagents are optimized for RNA isolation (low pH). For optimal DNA isolation, use samples freshly lysed in TRIzol®/TRI Reagent® (page 4). Freeze sample lysate (-80°C) for later processing.

Notes:

[1] RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol based reagents.*

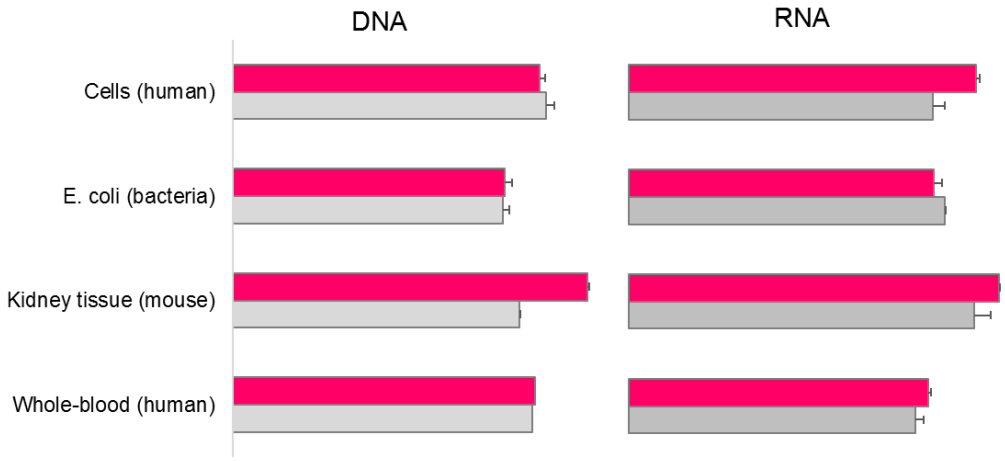
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility. Follow applicable federal, state and local regulations for phenol waste disposal.

Product Description

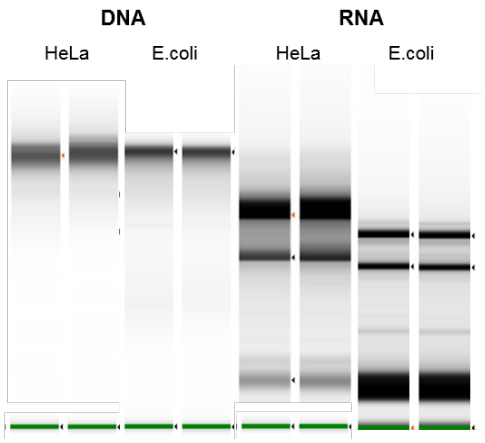
The **Direct-zol™ DNA/RNA** kits provide an innovative method for the purification of DNA and total RNA from a variety of samples freshly lysed in TRIzol® or similar[1], including animal cells, tissue, bacteria, yeast, plant, biological liquids and etc.

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.

One input. One column.
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).

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

Notes:

[1] TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol based reagents.*

Product Workflow:

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

Reagent Preparation:

- ✓ Before use, 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).
- ✓ 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).

Protocol:

This protocol consists of three parts:
(I) Sample Preparation, (II) RNA Purification and (III) DNA Purification

(I) Sample Preparation[1]:

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

Cells (Animal)

Lyse pelleted cells[3] in an appropriate volume of TRIzol® or similar[2] and mix thoroughly (see table below). Incubate the lysate at room temperature for 1 hour. Centrifuge debris (if any) and transfer the supernatant into a nuclease-free tube. Proceed to RNA Purification (page 5) or freeze lysate for later processing.

Animal	Bacterial	Yeast	Plant	Add TRI Reagent®
10^5	-	-	-	100 µl
10^6	10^8	10^7	-	300 µl
$5x10^6$	$5x10^8$	$5x10^7$	-	600 µl

Tough-to-Lyse Samples (Animal or Plant Tissue, Gram (+) Bacteria, Yeast, *etc.*)[4]

For maximum yield, mechanical homogenization using high efficiency bead beating (i.e., DNA/RNA Shield™ Lysis Tube, Microbe #R1103 and/or Tissue #R1105) and a high-speed homogenizer[5] is recommended. For samples already in TRIzol® or similar[2], a dounce, grinder or other homogenizers may be used. Centrifuge debris and transfer the supernatant into a nuclease-free tube. Proceed to RNA Purification (page 5) or freeze lysate for later processing.

Animal	Bacterial	Yeast	Plant	Add TRI Reagent®
10-25 mg	$5x10^8$	$5x10^7$	50-100 mg	> 600 µl

Biological Liquids (Whole-blood, Plasma, Serum, CSF, Buffy Coat, *etc.*)

Add 3 volumes TRIzol® or similar[2] to each liquid sample, mix well and incubate the lysate at room temperature for 1 hour. Centrifuge debris and transfer the supernatant into a nuclease-free tube. Proceed to RNA Purification (page 5) or freeze lysate for later processing.

For detailed processing information, refer to the reagent product manual (e.g., TRIzol®, TRI Reagent® or similar).

Notes:

[1] Sample preparation is a guideline for processing various sample types in TRIzol® or similar[2] prior to DNA/RNA purification. 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[2]).

[2] TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

[3] For cells suspensions, add 3 volumes of TRIzol® or similar[2] to each cell suspension. For direct lysis in a dish, add 100 µl TRIzol® for each cm^2 of culture surface area.

[4] Tissue sample should not exceed 10% of the TRIzol® volume used for homogenization.

[5] FastPrep®-24 (MP Biomedicals) or Precellys 24 Homogenizer (Bertin Instruments).

Notes:

[1] After adding ethanol to the lysate, mixture should be clear and free of debris, cloudiness or precipitation prior to loading into the column. Precipitation indicates inefficient lysis (i.e., too much sample).

[2] To process samples >700 µl, reload the column and repeat Step 2. Alternatively, a vacuum manifold can be used (for Step 2 only). After using the vacuum manifold, centrifuge the column to ensure complete removal of the TRIzol® sample and proceed with Step 3 of the protocol as stated.

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

[4] Before use, add ethanol to the buffer concentrate (see Reagent Preparation, page 4).

[5] For highly concentrated RNA and/or DNA, use ≥25 µl elution.

(II) 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 of ethanol (95%-100%) to the supernatant and mix thoroughly[1].

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

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. Carefully, transfer the column into a 1.5 ml nuclease-free tube (not provided).

5. To elute RNA[5], add 50 µl of **DNase/RNase-Free Water** directly to the column matrix and centrifuge. Keep the column and transfer it into a **new** collection tube for DNA Purification, step 6.

   RNA can be used immediately or stored frozen.

(III) 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 1.5 ml nuclease-free tube (not provided). 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 1.5 ml nuclease-free tube (not provided).

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

   DNA can be used immediately or stored frozen.

Appendix

Samples stored in DNA/RNA Shield™

Add 3 volumes TRIzol® or similar[1] to each sample homogenate in DNA/RNA Shield™ (3:1) and mix thoroughly. To remove particulate debris, centrifuge (12,000 *x g* for 1 minute) and transfer the supernatant into a nuclease-free tube (not provided). Proceed to RNA Purification followed by DNA Purification (page 5).

RNA purification from aqueous phase after TRIzol® extraction

For samples that have already been phase separated, simply transfer the aqueous phase[2] (top layer) containing RNA into a nuclease-free tube (not provided), add an equal volume ethanol (95-100%) and mix thoroughly. Proceed to RNA Purification (page 5, step 2).

RNA extraction from tissue samples stored in RNA*later*™

Remove tissue from RNA*later*™ using forceps. Eliminate any excess reagent or crystals that may have formed and proceed immediately to Sample Preparation, Tough-to-Lyse Samples (page 4).

Acetone precipitation of proteins

1. Add 4 volumes of cold acetone (-20 ºC) to the flow-through with ethanol obtained after RNA binding in the RNA Purification protocol (page 5, step 2).
2. Incubate samples for 30 minutes on ice.
3. Centrifuge at top speed for 10 minutes. Keep the protein pellet and carefully, discard the supernatant.
4. Add 400 µl ethanol (95-100%) to the pellet. Centrifuge at top speed for 1 minute and discard the supernatant.
5. Air-dry protein pellet for 10 minutes at room temperature.
6. Resuspend and vortex the pellet in a buffer appropriate for the downstream application.

   For example: SDS-PAGE sample loading buffer.

Notes:

[1] TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

[2] RNA from the aqueous phase can also be purified with the RNA Clean & Concentrator kit, #R1015.

Ordering Information:

Product Description	Kit Size	Catalog No.
Direct-zol™ DNA/RNA Miniprep	10 preps	R2080T
Direct-zol™ DNA/RNA Miniprep	50 preps	R2080
Direct-zol™ DNA/RNA Miniprep (TRI Reagent® included)	50 preps	R2081

For Individual Sale	Amount	Catalog No.
TRI Reagent®	50 ml 200 ml	R2050-1-50 R2050-1-200
RNA Prep Buffer	25 ml 100 ml	R1060-2-25 R1060-2-100
RNA Wash Buffer (concentrate)	12 ml 48 ml	R1003-3-12 R1003-3-48
DNase/RNase-Free Water	4 ml 6 ml	W1001-4 W1001-6
Direct-zol™ DNA Wash 1 (concentrate)	24 ml	R2080-1-24
Direct-zol™ DNA Wash 2	40 ml	R2080-2-40
Direct-zol™ DNA Elution Buffer	4 ml	R2080-3-4
Zymo-Spin™ IIC Columns	50 250	C1011-50 C1011-250
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-100
DNA/RNA Shield™ Lysis Tube (Microbe)	50	R1103
DNA/RNA Shield™ Lysis Tube (Tissue)	50	R1105

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