ZYMO RESEARCH

RNA Purification Made Simple™

RNA Clean & Concentrator™-96

Clean-up RNA from any sample

Highlights

- High-throughput, 96-well plate clean-up of total RNA (including small/microRNAs) from any enzymatic reaction, aqueous phase following TRIzol® extraction, in vitro transcription products, etc.
- Ultra-pure RNA is ready for Next-Gen Sequencing, RT-qPCR, etc.

Catalog Numbers:
R1080

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

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Product Contents

RNA Clean & Concentrator™-96	R1080 (2 x 96 prep)
RNA Binding Buffer	100 ml
RNA Prep Buffer	25 ml (x4)
RNA Wash Buffer (concentrate)[1]	24 ml (x2)
DNase/RNase-Free Water	6 ml
Zymo-Spin™ I-96 Plate	2
Collection Plate	2
Elution Plate	2
96-Well Plate Cover Foil	4
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Sources –** Enzymatic reactions (e.g., DNase I treated RNA), the aqueous phase following TRIzol®/chloroform or similar[1] extraction, in vitro transcriptions, etc.
- **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 –** 10 µg total RNA per well (**Zymo-Spin™ I-96 Plate**
- **Elution Volume –** ≥ 10 µl **DNase/RNase-Free Water**.
- **Equipment Needed** (user provided) **–** Centrifuge with 96-well plate carrier.

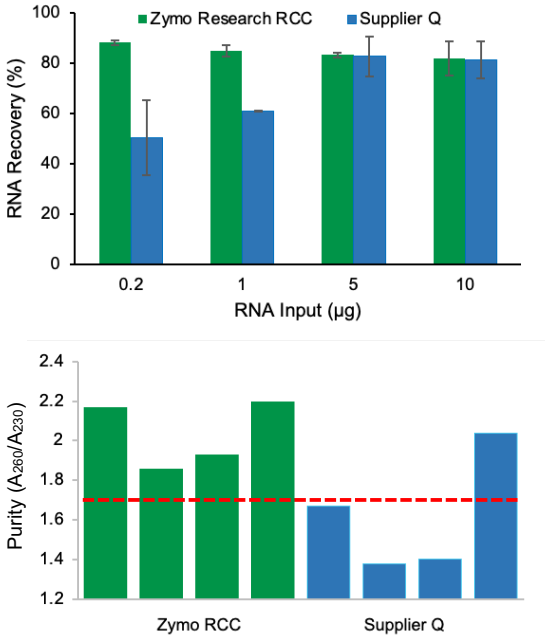
1 TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.

Product Description

The **RNA Clean & Concentrator™-96** kit provides a simple and reliable method for high throughput, 96-well plate clean-up of up to 10 µg/well of high-quality, NGS-ready RNA. The ~30-minute procedure is based on the use of a unique single-buffer system and **Zymo-Spin™** plate technology.

The kit allows for efficient RNA clean-up with the supplied **Zymo-Spin™ I-96 Plate**. RNA is washed, then eluted and concentrated into ≥ 10 µl of DNase/RNase-free Water. The highly concentrated, purified RNA is suitable for all subsequent analyses and molecular manipulations.

Consistent Recovery and Ultra-pure Total RNA



(top) Increasing amounts of RNA was cleaned up using the **RCC™** kit and a Supplier Q kit (n=2). **RCC™** provides higher yields and more consistent recovery when compared to the Supplier Q Kit.
(bottom) RNA was cleaned using the **RCC™** kit and a Supplier Q kit (n=4). RNA purity (measured by A260/230) was greater than 1.8 for the **RCC™** kit but not for the Supplier Q kit.

Protocol

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

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

(II) Total RNA Clean-up

- ✓ RNA species ≥ 17 nt will be recovered.
- ✓ Perform all steps at room temperature and centrifugation at 3,000-5,000 x g for 5 minutes, unless specified.
- ✓ For DNA-free RNA (optional), perform **DNase I** treatment before or during clean-up (page 6).
- ✓ Do not use the **96-Well Cover Foil** on the spin-plate during the RNA Clean-up. If necessary, use an Air Permeable Sealing Cover (#C2011-8); sold separately.

1. Add 2 volumes **RNA Binding Buffer** to each sample[1] and mix.

   Example: Mix 100 µl buffer and 50 µl sample.

2. Add an equal volume of ethanol[2] (95-100%) and mix.

   Example: Add 150 µl ethanol.

3. Transfer the sample to each well of the **Zymo-Spin™ I-96 Plate**[3] mounted on a **Collection Plate** and centrifuge. Discard the flow-through.

   Optional: At this point, in-column **DNase I** treatment can be performed (page 6).

4. Add 400 µl/well **RNA Prep Buffer** and centrifuge. Discard the flow-through.

5. Add 700 µl/well **RNA Wash Buffer** and centrifuge. Discard the flow-through.

6. Add 400/well µl **RNA Wash Buffer** to the column and centrifuge to ensure complete removal of the wash buffer. Carefully, mount the plate on an **Elution Plate**.

7. Add ≥ 10 µl/well **DNase/RNase-Free Water** directly to the matrix and centrifuge.

   The eluted RNA can be used immediately or stored frozen.

   Use the **96-Well Cover Foil** to prevent the eluate from evaporation.

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1 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).
2 Alternatively, if the sample consists of RNA species 17-200 nt only, use 1.5 volumes of ethanol (95-100%).
3 To process samples >700 µl, **Zymo-Spin™** well/plate may be reloaded.

Appendices

DNase I Treatment

- ✓ For DNA-free RNA, **DNase I** treatment can be performed using **DNase I Set** (E1010; 50 reactions) and RNA Wash Buffer (concentrate) (D1003-3-6); materials sold separately.
- ✓ Perform all steps at room temperature and centrifugation at 3,000-5,000 x g for 5 minutes, unless specified.

DNase I treatment before RNA clean-up

For each sample to be treated, prepare 50 µl **DNase I Reaction Mix** in an RNase-free tube (not provided) and mix by gentle inversion. Then incubate at room temperature (20-30°C) for 15 minutes and proceed with the RNA Clean-up protocol, page 5.

DNase I Reaction Mix	
RNA sample (≤ 10 µg; volume adjusted with water or TE buffer)	40 µl
DNase I (reconstituted; 1 U/ul)[1,2]	5 µl
DNA Digestion Buffer	5 µl

In-column DNase I treatment

1. Following RNA binding step (page 5, step 3), add 400 µl **RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (not provided) and mix by gentle inversion. Then add 40 µl directly into the matrix of each well and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the RNA Clean-up protocol (page 5, step 4).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/ul)[1,2]	5 µl
DNA Digestion Buffer	35 µl

1 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.
2 Reconstitute lyophilized **DNase I** (#E1009-A; 250 U) with 275 µl **DNase/RNase-Free Water** and mix by gentle inversion. Store frozen aliquots.

RNA clean-up from aqueous phase after TRIzol®/chloroform extraction

Following TRIzol®/chloroform or similar* extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided). Add 1 volume of ethanol (95-100%) to 1 volume of aqueous phase[1] (1:1) and mix well. Then proceed with the RNA Clean-up protocol, page 5, step 3.

RNA clean-up from samples in DNA/RNA Shield™

✓ Perform all steps at room temperature and centrifugation steps at 3,000-5,000 x g for 30 seconds, unless specified.

1. If frozen, thaw samples to room temperature (20-30°C) and centrifuge debris (if any). Transfer the cleared sample into an RNase-free tube (not provided).
2. Add 1 volume of ethanol (95-100%) to 1 volume of the **DNA/RNA Shield™** sample[1] and mix well.

   Example: 50 µl buffer and 50 µl sample.
3. Continue with the RNA Clean-up protocol, page 5, step 3.

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* TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.
1 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).

Ordering Information

Product Description	Catalog No.	Size
RNA Clean & Concentrator™-96	R1080	2 x 96 preps.

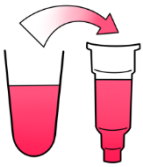
Individual Kit Components	Catalog No.	Amount
RNA Binding Buffer	R1013-2-25 R1013-2-50 R1013-2-100	25 ml 50 ml 100 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 ml
Zymo-Spin™ I-96 Plate	C2004	2
Collection Plate	C2002	2
Elution Plate	C2003	2
96-Well Plate Cover Foil	C2007-2 C2007-4	2 4
DNase/RNase-Free Water	W1001-6 W1001-10	6 ml 10 ml
DNase I Set (250 U DNase I (lyophilized) supplied with DNA Digestion Buffer, 4 ml)	E1010	1

Complete Your Workflow

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

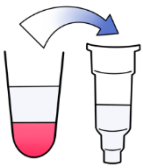
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ 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

Notes

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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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