ZYMO RESEARCH

RNA Purification Made Simple™

RNA Clean & Concentrator™-100

Clean-up RNA from any sample

Highlights

- Quick, 15-minute 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:
R1019

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

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Product Contents

RNA Clean & Concentrator™-100	R1019 (25 prep)
RNA Binding Buffer	100 ml
RNA Prep Buffer	10 ml
RNA Wash Buffer (concentrate)[1]	6 ml
DNase/RNase-Free Water	10 ml
Zymo-Spin™ V-E Columns (w/ reservoir)	25
Collection Tubes	50
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 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 –** 1 mg total RNA (**Zymo-Spin™ V-E Column**).
- **Elution Volume –** ≥ 100 µl **DNase/RNase-Free Water**.
- **Equipment Needed** (user provided) **–** Vacuum manifold, microcentrifuge.
- **Chemical Tolerance –** ≤5% Triton X-100, ≤5% Tween-20, ≤5% Sarkosyl, ≤ 0.1% SDS. Also compatible with ≤90% Sucrose, ≤90% Formamide, and ≤2% Formaldehyde.

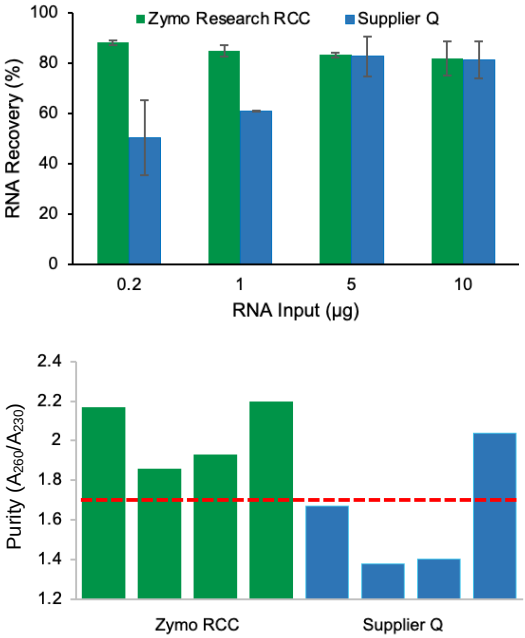
1 TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.

Product Description

The **RNA Clean & Concentrator™-100** kit provides a simple and reliable method for the rapid preparation of up to 1 mg of high-quality, NGS-ready RNA. This 15 minute procedure is based on the use of a unique single-buffer system and Zymo-Spin™ column technology that allows for selective recovery of total RNA (> 17 nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt).

The procedure is easy: Add binding buffer to your sample, then bind, wash and elute ultra-pure RNA. The RNA can be eluted from the **Zymo-Spin™ V-E Column** in ≥ 100 µl of 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) Total RNA Clean-up.

(I) Buffer Preparation

- ✓ Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 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 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ For DNA-free RNA (optional), perform **DNase I** treatment before clean-up (page 6).

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

   Example: Mix 1 ml buffer and 0.5 ml sample.

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

   Example: Add 1.5 ml ethanol.

3. Transfer the sample to the **Zymo-Spin™ V-E Column w/ Reservoir** mounted on a vacuum manifold and start vacuum[1]. Discard the flow-through. Place column into a **Collection Tube**.

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

5. Add 400 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

6. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 1 minute ensure complete removal of the wash buffer. Carefully, transfer the column into a RNase-free tube (not provided).

7. Add ≥ 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   The eluted RNA can be used immediately or stored frozen.

1 Set vacuum source at ≥ 500 mm Hg.

Appendices

DNase I Treatment

- ✓ For DNA-free RNA, **DNase I** treatment can be performed using **DNase I Set** (E1010; qty: 2) and RNA Wash Buffer (concentrate) (D1003-3-6); materials sold separately.
- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

DNase I treatment before RNA clean-up

For each sample to be treated, prepare 200 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 (≤ 250 µg; volume adjusted with water or TE buffer)	160 µl
DNase I (reconstituted; 1 U/ul)[1,2]	20 µl
DNA Digestion Buffer	20 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 each vial of lyophilized **DNase I** (#E1009-A; 250 U) with 275 µl **DNase/RNase-Free Water** and mix by gentle inversion. Store frozen aliquots.

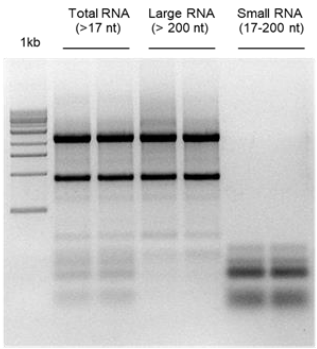
Purification of Small and Large RNAs into Separate Fractions

- ✓ Perform all steps at room temperature and centrifugation steps at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ This protocol requires two column per prep.

1. Prepare adjusted **RNA Binding Buffer** (as needed). Mix an equal volume of buffer and ethanol (95-100%).
   Example: Mix 500 µl buffer and 500 µl ethanol.
2. Add 2 volumes of the adjusted buffer to the sample[1] and mix.
   Example: Mix 1 ml adjusted buffer and 0.5 ml sample.
3. Transfer the mixture to the **Zymo-Spin™ Column**[2] and centrifuge. **Save the flow-through!**

4. **Small RNAs (17-200 nt)**
   are in the flow-through
   a. Add 1 volume ethanol and mix.
      Example: Add 1.5 ml ethanol to 1.5 ml sample.
   b. Transfer the mixture to a **new column** and centrifuge. Discard the flow-through.
   c. Proceed with the RNA Clean-up protocol, page 5, step 4.

4. **Large RNAs (> 200 nt)**
   are retained in the column
   a. Proceed with the RNA Clean-up protocol, page 5, step 4.



RNA Clean & Concentrator™ allows for clean-up of total RNA (> 17 nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt).

1 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).
2 To process samples >700 µl, **Zymo-Spin™** columns may be reloaded.

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 10,000-16,000 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.

cDNA Clean-Up following Reverse Transcription (RT)

The **RNA Clean & Concentrator** can be used to effectively clean and concentrate first-strand cDNA following reverse transcription (RT) and hydrolysis. The **RNA Binding Buffer** will neutralize the hydrolysis reaction and the recovered cDNA may be used directly for microarray analysis, etc.

Hydrolysis Reaction: To each 30-50 µl RT reaction, add 10 µl 0.5 M EDTA and 10 µl 1 M NaOH. Then mix and incubate at 65°C for 15 minutes. Proceed to the Total RNA Clean-Up protocol, page 5.

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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™-100	R1019	25preps.

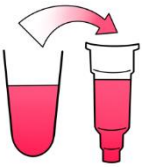
Individual Kit Components	Catalog No.	Amount
RNA Binding Buffer	R1013-2-50 R1013-2-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-24	6 ml 24 ml
Zymo-Spin™ V-E Columns (w/ reservoir)	C1029-25	25
Collection Tubes	C1001-50 C1001-500	50 500
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:

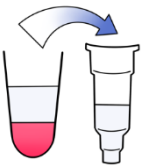
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ 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

Format Compatibility

To adjust binding capacity, simply replace the provided columns with the formats indicated below and follow their respective kit protocol.

Format	Zymo-Spin™ I & IC	Zymo-Spin™ II & IICR	Zymo-Spin™ V-E	Zymo-Spin™ I-96 Plate
Item Image				
Kit Name	RCC™-5	RCC™-25	RCC™-100	RCC™-96
Capacity	10 µg / prep.	50 µg / prep.	1 mg / prep.	10 µg / prep.
Elution Vol.	≥ 6 µl	≥ 25 µl	≥ 100 µl	≥ 10 µl
Column Cat. Nos.	C1003-50, C1004-50	C1008-50, C1078-50	C1024-25, C1029-25	C2004

Notes

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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