



ZYMO RESEARCH

RNA
Purification
Made Simple

RNA Clean & Concentrator™ -5

Clean-up RNA from any sample

Highlights

- Quick, 5-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. *DNase I is included.*

Catalog Numbers:

R1013, R1014 (supplied with DNase I Set)

R1015, R1016



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	01
Specifications	02
Product Description	03
Protocol	04
(I) Buffer Preparation	04
(II) Total RNA Clean-up	05
Appendices	06
DNase I Treatment	06
Purify Small and Large RNAs in Separate Fractions	07
RNA Clean-up from TRIzol Aqueous Phase	08
RNA Clean-up from DNA/RNA Shield™	08
cDNA Clean-Up	08
Ordering Information	09
Complete Your Workflow	10
Format Compatibility	11
Notes	12
Guarantee	13

Product Contents

RNA Clean & Concentrator™-5	R1013 (50 prep)	R1014 (200 prep)	R1015 (50 prep)	R1016 (200 prep)
RNA Binding Buffer	25 ml	100 ml	25 ml	100 ml
RNA Prep Buffer	25 ml	100 ml	25 ml	100 ml
RNA Wash Buffer (concentrate)	24 ml	24 ml (x3)	24 ml	24 ml (x3)
DNase I ² (lyophilized)	250 U	250 U (x4)	-	-
DNA Digestion Buffer	4 ml	16 ml	-	-
DNase/RNase-Free Water	4 ml	10 ml	4 ml	10 ml
Zymo-Spin IC Columns	50	200	50	200
Collection Tubes	50	200	50	200
Instruction Manual	1	1	1	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.

2 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water** (R1013, R1014) and mix by gentle inversion. Store frozen aliquots.

#E1009-A (250 U), add 275 µl water

#E1009-A-S (50 U), add 55 µl water

Specifications

- **Sample Sources** – Enzymatic reactions (e.g., DNase I treated RNA), the aqueous phase following TRIzol®/chloroform or similar¹ 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 (**Zymo-Spin™ IC Column**)
- **Elution Volume** – $\geq 6 \mu\text{l}$ **DNase/RNase-Free Water**
- **Equipment Needed** (user provided) – Microcentrifuge
- **Chemical Tolerance** – $\leq 5\%$ Triton X-100, $\leq 5\%$ Tween-20, $\leq 5\%$ Sarkosyl, $\leq 0.1\%$ SDS. Also compatible with $\leq 90\%$ Sucrose, $\leq 90\%$ Formamide, and $\leq 2\%$ Formaldehyde.

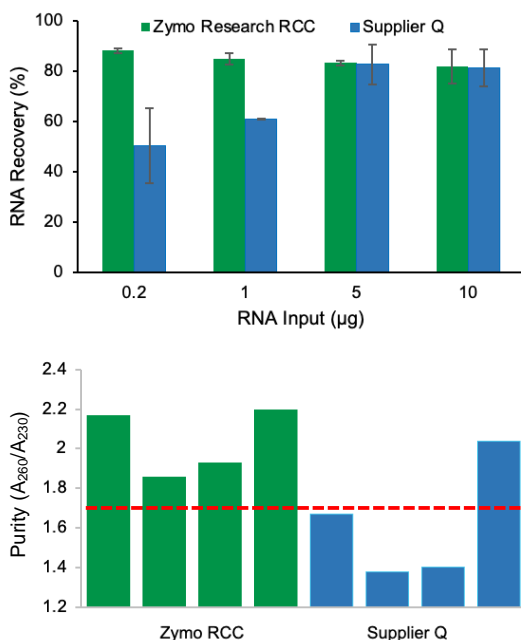
¹ TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.

Product Description

The **RNA Clean & Concentrator™-5** kit provides a simple and reliable method for the rapid preparation of up to 10 µg of high-quality, NGS-ready and DNA-free (R1013, R1014) RNA. This 5 minute procedure is based on the use of a unique single-buffer system and Zymo-Spin™ technology that allows for selective recovery of total RNA (> 17nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt).

The procedure is easy: Add binding buffer and ethanol to your sample, then bind, wash and elute ultra-pure RNA. The RNA can be eluted from the **Zymo-Spin™ IC Column** in as little as ≥ 6 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/A₂₃₀) 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 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
- ✓ Prior to use, reconstitute the lyophilized **DNase I** with **DNase/RNase-Free Water** and mix by gentle inversion. Store frozen aliquots.
#E1009-A (250 U), add 275 μ l **water**
#E1009-A-S (50 U), add 55 μ l **water**
#E1011-A (1500 U), add 1,500 μ l **water**

(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 (recommended) or during clean-up (page 6).

1. Add 2 volumes **RNA Binding Buffer** to each sample¹ and mix.

Example: Mix 100 μ l buffer and 50 μ l sample.

2. Add an equal volume of ethanol² (95-100%) and mix.

Example: Add 150 μ l ethanol.

3. Transfer the sample to the **Zymo-Spin™ IC Column**³ in a **Collection Tube** and centrifuge. Discard the flow-through.

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

4. Add 400 μ l **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
5. Add 700 μ l **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
6. Add 400 μ l **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 15 μ l **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use ≥ 6 μ l elution.

The eluted RNA can be used immediately or stored frozen.

1 To minimize pipetting error, adjust the sample volume to 50 μ l (minimum).

2 Alternatively, if working exclusively with RNA fragments 17-200 nt in length, use 1.5 volumes of 95-100% ethanol.

3 To process samples >700 μ l, **Zymo-Spin™** columns may be reloaded.

Appendices

DNase I Treatment

- ✓ 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 (Recommended)

For each sample to be treated, prepare 50 μ l **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 ($\leq 10 \mu\text{g}$; volume adjusted with water or TE buffer)	40 μ l
DNase I (reconstituted; 1 U/ μ l) ¹	5 μ l
DNA Digestion Buffer	5 μ l

In-column DNase I treatment

1. Following RNA binding step (page 5, step 3), add 400 μ l **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 μ l directly into column matrix 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/ μ l) ¹	5 μ l
DNA Digestion Buffer	35 μ l

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.

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 columns per prep.

1. Prepare adjusted **RNA Binding Buffer** (as needed). Mix an equal volume of buffer and ethanol (95-100%).

Example: Mix 50 µl buffer and 50 µl ethanol.

2. Add 2 volumes of the adjusted buffer to the sample¹ and mix.

Example: Mix 100 µl adjusted buffer and 50 µl 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.

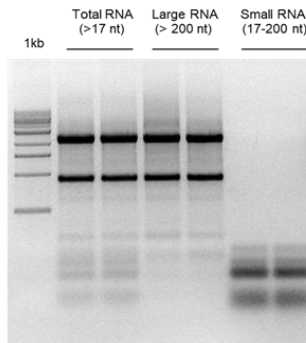
Example: Add 150 µl ethanol to 150 µl sample.

- 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 phase¹ (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™** sample¹ 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.

* 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™-5 (supplied with DNase I Set)	R1013 R1014	50 preps. 200 preps.
RNA Clean & Concentrator™-5	R1014 R1015	50 preps. 200 preps.

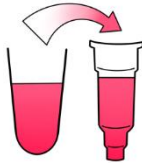
Individual Kit Components	Catalog No.	Amount
RNA Binding Buffer	R1013-2-25 R1013-2-50	25 ml 50 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500	50 500
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 set

Complete Your Workflow

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

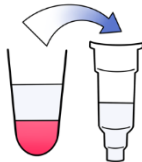
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

- ✓ 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):






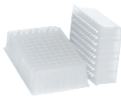
RNA Clean & Concentrator kit	
#R1013-R1014	DNase I Set included

- ✓ 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.

TM Trademarks of Zymo Research Corporation
RNA Clean & Concentrator[®] is a registered trademark of Zymo Research Corporation. Other trademarks: TRI Reagent[®], TRIzol[®] and RNAzol[®] (Molecular Research Center, Inc.), QIAzol[®] (Qiagen GmbH), TriPure[™] (Roche, Inc.), TriSure[™] (Bioline Ltd.), RNAlater[®] (Ambion, Inc.), Bioanalyzer (Agilent Technologies, Inc.).



ZYMO RESEARCH

The **BEAUTY** of **SCIENCE** is to Make Things **SIMPLE**®



tech@zymoresearch.com



www.zymoresearch.com



Toll Free: (888) 882-9682