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.

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Table of Contents

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 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 (**Zymo-Spin™ IC Column**)
- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water**
- **Equipment Needed** (user provided) **–** Microcentrifuge

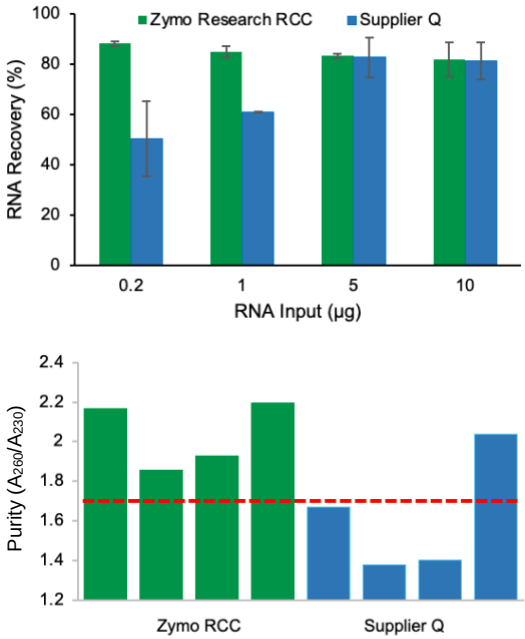
1 TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.

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 A260/230) 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 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 µl **water**
  #E1009-A-S (50 U), add 55 µl 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 or during clean-up (page 6).

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

5. Add 700 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 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

   The eluted RNA can be used immediately or stored frozen.

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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™** 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

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]	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 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/ul)[1]	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.

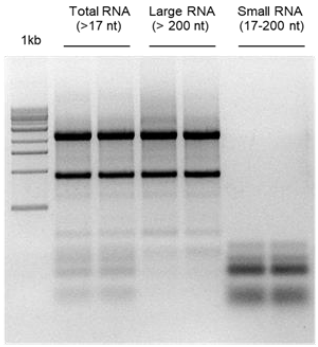
Purification of Small and Large RNAs into Separate Fractions

- ✓ 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 50 µl buffer and 50 µl ethanol.
2. Add 2 volumes of the adjusted buffer to the sample[1] 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 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.

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

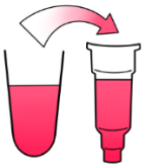
Individual Kit Components	Catalog No.	Amount
RNA Binding Buffer	R1013-2-25 R1013-2-50	25 ml 50 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500	50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

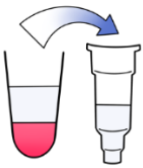
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ 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

Notes

Notes

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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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