ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

RNA Clean & Concentrator™-5

Catalog Nos. **R1013 & R1014** (supplied with DNase I)
R1015 & R1016

Highlights

- Quick, 5-minute recovery of ultra-pure RNA (≥17 nt) from enzymatic reactions (DNase), *in vitro* transcription products and etc.
- Compatible with aqueous phase following TRIzol® extraction and samples in DNA/RNA Shield™.
- High-quality RNA eluted in ≥6 µl is ready for RT/PCR, Next-Gen sequencing, hybridization, etc.

Contents

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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.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

RNA Clean & Concentrator™-5 (Kit Size)	R1013 (50)	R1014 (200)	R1015 (50)	R1016 (200)	Storage Temperature
RNA Binding Buffer	25 ml	100 ml	25 ml	100 ml	Room Temp.
RNA Prep Buffer	25 ml	100 ml	25 ml	100 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	24 ml	3 x 24 ml	24 ml	3 x 24 ml	Room Temp.
DNase I[2] (lyophilized)	1 x 250 U	4 x 250 U	-	-	-20ºC (reconstituted)
DNA Digestion Buffer	4 ml	16 ml	-	-	Room Temp.
DNase/RNase-Free Water	4 ml	10 ml	4 ml	10 ml	Room Temp.
Zymo-Spin™ IC Columns	50	200	50	200	Room Temp.
Collection Tubes	50	200	50	200	Room Temp.
Instruction Manual	1	1	1	1	-

Note - 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.

[1] Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

[2] Prior to use, reconstitute the lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:

#**E1009-A (250 U)**, add 275 µl **water**

#E1009-A-S (50 U), add 55 µl water

Specifications

- **Sample Types** – Enzymatic reactions such as DNase I treated RNA and *in vitro* transcription products. Also compatible with the aqueous phase following TRIzol®/chloroform or similar[3] extraction and samples in DNA/RNA Shield™.
- **Binding Capacity** – 10 µg total RNA
- **Size Limits** – From 17 nt to ~23 kb
- **Elution Volume** – ≥6 µl
- **Purity** – RNA is ready for RT/PCR, Next-Gen sequencing, hybridization, etc. (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8)
- **RNA Storage** – RNA is eluted with nuclease-free water and can be stored at ≤70ºC. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Required Equipment** – Microcentrifuge

Notes:

[3] Compatible with: TRIzol®, TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and other *acid-guanidinium-phenol* reagents.

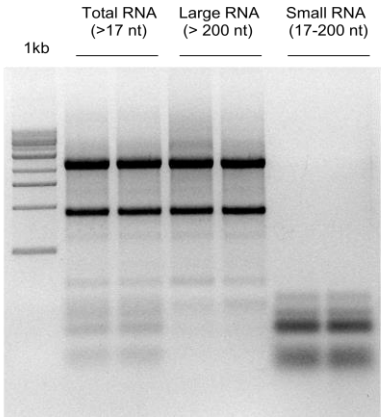
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution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

Product Description

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

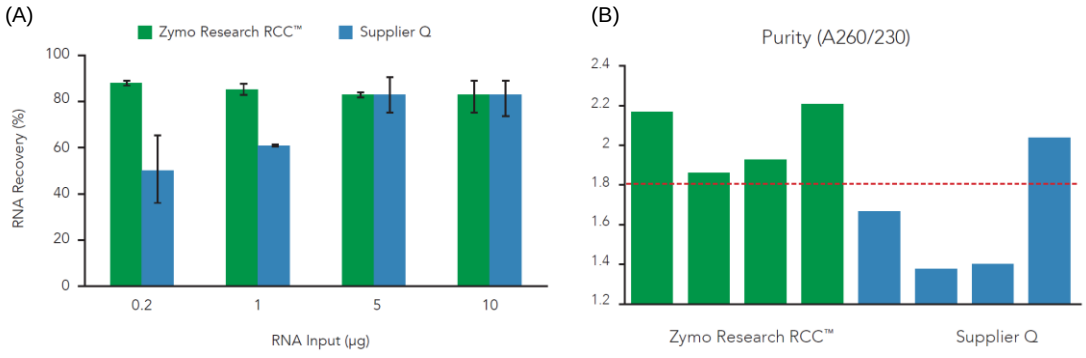
The procedure is easy: Add binding buffer and ethanol to your sample, then bind, wash and elute ultra-pure RNA. The RNA can be eluted from the **Zymo-Spin™ IC Column** in as little as ≥6 µl of nuclease-free water. The highly-concentrated, purified RNA is suitable for all subsequent analyses and molecular manipulations.
The entire procedure typically takes about 5 minutes.

Purification of Small and Large RNAs into Separate Fractions



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

Consistent Recovery and Ultra-pure Total RNA



(A) 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.
(B) RNA was cleaned-up 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.

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

Note:

For purification of DNA see the **DNA Clean & Concentrator™-5** and **-25** (Catalog Nos. D4013, D4014, D4033, D4034).

Ensure the RNA isolation procedure is performed in an RNase-free environment.

Reagent Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
- ✓ Prior to use, reconstitute the lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
  #**E1009-A (250 U)**, add 275 µl **water**
  #E1009-A-S (50 U), add 55 µl water

Protocol

- ✓ Perform all steps at room temperature and centrifugation at 10,000 – 16,000 *x g* for 30 seconds, unless specified.
- ✓ RNA species ≥17 nt will be recovered.
- ✓ For DNA-free RNA, perform DNase I treatment prior or during the clean-up protocol (page 4).

Notes:

[1] Adjust the sample volume to 50 µl (minimum).

[2] To process samples >800 µl, **Zymo-Spin™** columns may be reloaded.

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 (95-100%) and mix.

   Example: Add 150 µl ethanol.

3. Transfer the sample[2] to the **Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge. Discard the flow-through.
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 nuclease-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 at -70°C.

Appendix A: DNase I treatment

✓ Perform all steps at room temperature and centrifugation at 10,000 – 16,000 *x g* for 30 seconds.

There are two methods of performing the DNase I digestion: (I) before the clean-up and (II) during the clean-up (in-column). Choose an appropriate method for your application below:

(I) Before clean-up

The DNase digestion procedure can be performed using the **DNase I Set** (E1010; provided with R1013, R1014)[1].

1. For each sample to be treated, prepare **DNase I reaction mix** in an RNase-free tube (not provided). Mix well by gentle inversion:

RNA sample (≤10 µg)	
volume adjusted with water or TE buffer	40 µl
DNase I	5 µl
DNA Digestion Buffer	5 µl
	50 µl

2. Incubate at room temperature (20-30ºC) for 15 minutes. Then start with RNA purification protocol (page 3, step 1).

(II) In-column

1. Following the RNA binding step (page 3, step 3), prewash the column with 400 µl **RNA Wash Buffer**. 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). Mix well by gentle inversion:

DNase I	5 µl
DNA Digestion Buffer	35 µl

3. Add 40 µl reaction mix directly to the column matrix. Incubate at room temperature (20-30ºC) for 15 minutes. Then continue with the RNA purification protocol (page 3, step 4).

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

Appendix B: Purification of small and large RNAs into separate fractions

- ✓ Perform all steps at room temperature and centrifugation 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 sample and mix.

   Example: Mix 100 µl adjusted buffer and 50 µl sample.

3. Transfer the mixture to the **Zymo-Spin™ Column** and centrifuge.

Save the flow-through!

4. 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.	RNAs >200 nt are retained in the **column**. Continue with step 5.

5. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
6. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
7. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 1 minute to ensure complete removal of the wash buffer. Carefully, transfer the column into a nuclease-free tube (not provided).
8. 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 at -70°C.

Appendix C: RNA purification from aqueous phase after TRIzol® extraction

✓ Perform all steps at room temperature.

1. Following TRIzol®/chloroform or similar[1] extraction, carefully transfer the upper aqueous phase into a nuclease-free tube (not provided).
2. For each volume of the aqueous phase (as measured or estimated), add 1 volume ethanol (95-100%) and mix.
3. Continue with the RNA purification protocol (page 3, step 3).

Note:

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

Appendix D: RNA Purification from samples in DNA/RNA Shield™

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

Before starting, bring samples homogenized and stored in DNA/RNA Shield™ to room temperature (20-30°C).

1. Add 1 volume of **RNA Binding Buffer** to each volume of DNA/RNA Shield™ sample and mix well.

   Example: 100 µl buffer and 100 µl sample.
2. For any particulate removal, centrifuge lysate and transfer the supernatant into a nuclease-free tube (not provided).
3. Continue with the RNA Purification protocol (page 3, step 3).

Ordering Information

Product Description	Kit Size	Catalog No.
RNA Clean & Concentrator™-5	50 Preps. 200 Preps.	R1015 R1016
RNA Clean & Concentrator™-5 with DNase I Set	50 Preps. 200 Preps.	R1013 R1014
RNA Clean & Concentrator™-25	50 Preps. 100 Preps.	R1017 R1018
RNA Clean & Concentrator™-100	25 Preps.	R1019
ZR-96 RNA Clean & Concentrator™	2x 96 Preps.	R1080

For Individual Sale	Amount	Catalog No.
RNA Binding Buffer	25 ml 50 ml 100 ml 1000 ml	R1013-2-25 R1013-2-50 R1013-2-100 R1013-2-1000
RNA Prep Buffer	10 ml 25 ml 100 ml	R1060-2-10 R1060-2-25 R1060-2-100
RNA Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48
Zymo-Spin™ IC Columns	50 250	C1004-50 C1004-250
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000
DNase/RNase-Free Water	1 ml 4 ml 6 ml 10 ml	W1001-1 W1001-4 W1001-6 W1001-10
DNase I Set DNase I (250 U) & DNA Digestion Buffer (4 ml)	1 set	E1010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