



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## **RNA Clean & Concentrator™-5**

Catalog Nos. R1015 & R1016

### **Highlights**

- Quick (*5 minute*) method for cleaning and concentrating RNA.
- Ideal for purification of RNA from aqueous phase following an *acid phenol* extraction.
- *Fast-Spin* column technology allows RNA to be eluted into minimal volumes ( $\geq 6 \mu\text{l}$ ).
- Eluted RNA is ultra clean and ready for subsequent analysis and molecular manipulation.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

## Product Contents

<b>RNA Clean &amp; Concentrator™-5 (Kit Size)</b>	<b>R1015 (50 Preps.)</b>	<b>R1016 (200 Preps.)</b>	<b>Storage Temperature</b>
<b>RNA Binding Buffer</b>	25 ml	100 ml	Room Temp.
<b>RNA Prep Buffer</b>	25 ml	4x 25 ml	Room Temp.
<b>RNA Wash Buffer<sup>1</sup> (concentrate)</b>	12 ml	2x 24 ml	Room Temp.
<b>DNase/RNase-Free Water</b>	1 ml	4 ml	Room Temp.
<b>Zymo-Spin™ IC Columns</b>	50	200	Room Temp.
<b>Collection Tubes</b>	50	200	Room Temp.
<b>Instruction Manual</b>	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.

<sup>1</sup> Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1015) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1016) before use.

## Specifications

- **Sample Sources** – RNA fragments ( $\geq 17$  nucleotides): DNase treated RNA, *in vitro* transcription products, the aqueous phase following an *acid phenol* extraction methods (*e.g.*, TRI Reagent®) *etc.*
- **Format** – Spin column.
- **RNA Purity** – High quality RNA ( $A_{260}/A_{280} > 1.8$ ,  $A_{260}/A_{230} > 1.8$ ) suitable for all downstream RNA-based manipulations.
- **RNA Recovery** – Typically, RNA is eluted into as little as 6-10  $\mu$ l RNase-free water allowing for a highly concentrated sample. The RNA binding capacity of the supplied **Zymo-Spin™ IC Columns** is  $\sim 5 \mu$ g.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at  $\leq -70$  °C. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. 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. TRI Reagent is a registered trademark of Molecular Research Center.

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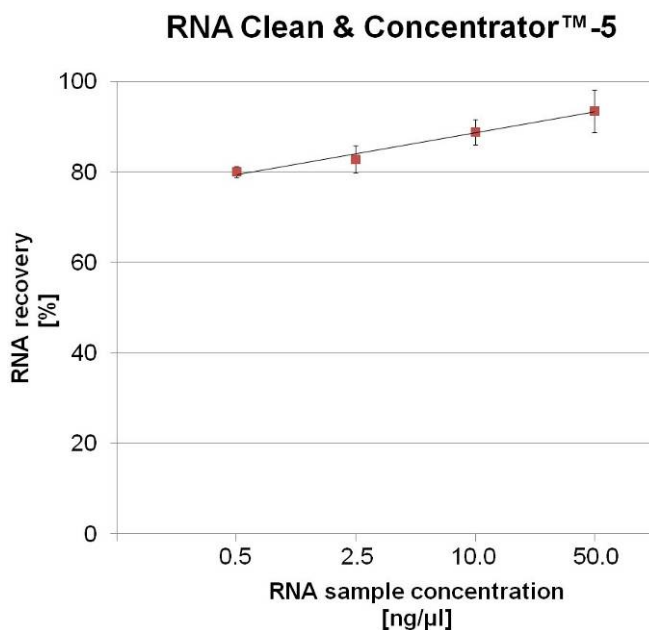
Toll Free: 1-888-882-9682 • Fax: 1-714-288-9643 • Web: [www.zymoresearch.com](http://www.zymoresearch.com) • E-mail: [info@zymoresearch.com](mailto:info@zymoresearch.com)

## Product Description

**RNA Clean & Concentrator™-5** provides a simple and reliable method for the rapid preparation of up to ~5 µg (per prep.) of high-quality RT-PCR-ready RNA. This simple procedure is based on the use of a unique single-buffer system and *Fast-Spin* column technology.

The procedure is easy: just add the buffer to your sample, adjust the conditions for binding by adding ethanol, and the cleaned RNA is then concentrated into ≥6 µl of RNase-free water using a **Zymo-Spin™ IC Column**. RNA fragments (≥17 bases) can be safely treated and recovered using this kit. The result is highly-concentrated, purified RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, *etc.*

*The entire procedure typically takes about 5 minutes.*



Concentration of diluted RNA samples (n = 3, total input = 1 µg RNA) using the **RNA Clean & Concentrator™-5**. RNA was eluted with 20 µl RNase-free water.

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

**Note:**

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

Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

## Buffer Preparation

Before starting, add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1015) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1016).

## Protocol

### General procedure<sup>1</sup>

*total RNA (>17nt)*

1. Add 2 volumes of **RNA Binding Buffer** to each volume of RNA sample<sup>3</sup> and mix well.
2. Add 1 volume ethanol (95-100%) to the mixture from *Step 1* (e.g., 200  $\mu$ l ethanol and 200  $\mu$ l mixture), and mix well.

OR

### small RNA elimination<sup>2</sup>

*total RNA (>200nt)*

- Mix 1 volume of **RNA Binding Buffer** with 1 volume ethanol (95-100%).
- Add 2 volumes of the adjusted buffer from *Step 1* to 1 volume of an RNA sample<sup>3</sup> (e.g., 200  $\mu$ l mixture and 100  $\mu$ l RNA) and mix well.

### Notes:

<sup>1</sup> RNA species  $\geq 17$  nt will be recovered.

<sup>2</sup> RNA species  $\leq 200$  nt will be removed.

<sup>3</sup> Minimal recommended sample volume is 50  $\mu$ l. Adjust samples  $< 50$   $\mu$ l with RNase-free water.

<sup>4</sup> **Optional DNase treatment:** Following *Step 3*, samples can be *in-column* or *in-tube* DNase treated. See **Appendices A** and **B**, respectively.

<sup>5</sup> RNase-free water is strongly recommended for RNA elution. TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) can also be used for elution if required by your experiment. Waiting for 1 to 2 minutes after adding the RNase-free water to the column matrix may increase RNA yield. Also, the yield may be increased by performing a second elution.

3. Transfer the mixture from *Step 2* to the **Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge at  $\geq 12,000 \times g$  for 1 minute<sup>4</sup>. Discard the flow-through.
4. Add 400  $\mu$ l **RNA Prep Buffer** to the column and centrifuge at  $\geq 12,000 \times g$  for 1 minute. Discard the flow-through.
5. Add 800  $\mu$ l **RNA Wash Buffer** to the column and centrifuge at  $\geq 12,000 \times g$  for 30 seconds. Discard the flow-through. Repeat the wash step with 400  $\mu$ l **RNA Wash Buffer**.
6. Centrifuge the **Zymo-Spin™ IC Column** in an emptied **Collection Tube** at  $\geq 12,000 \times g$  for 2 minutes. Remove the **Zymo-Spin™ IC Column** carefully from the **Collection Tube** and transfer it into an RNase-Free Tube.
7. Add  $\geq 6$   $\mu$ l of **DNase/RNase-Free Water**<sup>5</sup> directly to the column matrix and let stand for 1 minute at room temperature. Centrifuge at  $10,000 \times g$  for 30 seconds. The eluted RNA can be used immediately or stored at  $-70^\circ\text{C}$ .

**Ordering Information**

Product Description	Catalog No.	Kit Size
RNA Clean & Concentrator™-5	R1015	50 Preps.
	R1016	200 Preps.
RNA Clean & Concentrator™-25	R1017	50 Preps.
	R1018	100 Preps.

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

*Fast-Spin* column technology employed in ZR RNA isolation products efficiently removes the majority of DNA during RNA purification and is satisfactory for most RNA-based applications. However, if necessary, complete removal of DNA can be achieved by performing a DNase I digestion.

## **Appendix A**

### **In-Column DNase Digestion**

The DNase digestion procedure can be performed using any source of RNase-free DNase I together with its 10X reaction buffer (e.g., 100 U **RNase-free DNase I (1 U/μl) w/ 10X Reaction Buffer** – Zymo Research Cat. No. **E1007**). DNase I maintains activity in the **RNA Wash Buffer** provided in this kit.

1. Make the following DNase I cocktail (for each sample to be treated):

RNase-Free DNase I	3 μl (1 U/μl)
10X Reaction Buffer	3 μl
<b>RNA Wash Buffer</b>	24 μl

2. Following Step 3 of the RNA isolation protocol<sup>1</sup>, add 400 μl **RNA Wash Buffer** to the **Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge at ≥12,000 x *g* for 30 seconds. Discard the flow through.
3. Add 30 μl DNase I cocktail from *Step 1* above directly to the matrix of the **Zymo-Spin™ IC Column**. Keep the **Zymo-Spin™ IC Column** in the **Collection Tube**.
4. Incubate the column at 25-37°C for ≥15 minutes<sup>2</sup>, then centrifuge ≥12,000 x *g* for 30 seconds. Discard the flow-through.
5. Continue with Step 4 of the RNA isolation protocol<sup>3</sup>.

#### **Notes:**

<sup>1</sup> See page 3, Protocol – *Step 3*.

<sup>2</sup> The temperature optimum for DNase I activity is at 37 °C. An optimal incubation time may vary.

<sup>3</sup> See page 3, Protocol – *Step 4*.

## **Appendix B**

### **In-Tube DNase Digestion**

The procedure can be performed using any source of RNase-free DNase I together with its 10X reaction buffer (e.g., 100 U **RNase-Free DNase I (1 U/μl) w/ 10x Reaction Buffer** – Zymo Research Cat. No. **E1007**).

1. Make the following DNase I cocktail (for each sample to be treated):

RNase-Free DNase I	3 μl (1 U/μl)
10X Reaction Buffer	3 μl
<b>DNase/RNase-Free Water</b>	24 μl

2. Following Step 3 in the RNA isolation protocol<sup>1</sup>, add 400 μl **RNA Wash Buffer** to the **Zymo-Spin™ IC Column** and centrifuge at ≥12,000 x g for 30 seconds.
3. Transfer the **Zymo-Spin™ IC Column** into an RNase-free tube.
4. Add 30 μl DNase I cocktail from *Step 1* above directly to the matrix of the column and centrifuge at 500 x g 30 seconds. Keep the **Zymo-Spin™ IC Column** in the RNase-free tube.  
*Save the column and the flow-through in the RNase-free tube!*
5. Incubate at room temperature for ≥15 minutes<sup>2</sup>, then centrifuge ≥12,000 x g for 30 seconds.
6. Transfer the **Zymo-Spin™ IC Column** into a new **Collection tube**.
7. Add 120 μl **RNA Binding Buffer** to the 30 μl flow-through in the RNase-free tube (from *Step 5*) and mix well by pipetting.
8. Add 150 μl ethanol (95-100%) to the mixture from *Step 7*. Mix well by pipetting and reload onto the **Zymo-Spin™ IC Column** in a **Collection Tube**. Centrifuge at ≥12,000 x g for 30 seconds.
9. Continue with Step 4 of the RNA isolation protocol<sup>3</sup>.

#### **Notes:**

<sup>1</sup> See page 3, *Step 3*.

<sup>2</sup> The temperature optimum for DNase I activity is at 37 °C. An optimal incubation time may vary.

<sup>3</sup> See page 3, *Step 4*.

## RNA Purification Products - Zymo Research

Product	Description	Kit Size	Cat. No.
<b>Total RNA Isolation</b>			
ZR Whole-Blood RNA Kit™	Spin Column Format (up to 25 µg/prep.)	50 preps. 100 preps.	R1020 R1021
ZR-96 Whole-Blood RNA Kit™	96-Well Format (up to 25 µg/prep.)	2x96 preps.	R1022
ZR Viral RNA Kit™	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	R1034 R1035
ZR-96 Viral RNA Kit™	96-Well Format (up to 5 µg/well)	2x96 preps. 4x96 preps.	R1040 R1041
ZR Urine RNA Isolation Kit™	Spin Column Format (up to 5 µg/prep.)	50 preps.	R1039
Quick-RNA™ MicroPrep	Spin Column Format (up to 5 µg/prep.)	50 preps.	R1050
Quick-RNA™ MiniPrep	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	R1054 R1055
ZR RNA MicroPrep™	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	R1060 R1061
ZR RNA MiniPrep™	Spin Column Format (up to 25 µg/prep.)	50 preps. 200 preps.	R1064 R1065
Pinpoint™ Slide RNA Isolation System Kit I	Spin Column Format (up to 5 µg/prep.)	50 preps.	R1003
Pinpoint™ Slide RNA Isolation System Kit II	Spin Column Format (up to 5 µg/prep.)	50 preps.	R1007
ZR Fungal/Bacteria RNA MicroPrep™	Spin Column Format (up to 5 µg/prep.)	50 preps.	R2010
ZR Fungal/Bacteria RNA MiniPrep™	Spin Column Format (up to 25 µg/prep.)	50 preps.	R2014
ZR Plant RNA MiniPrep™	Spin Column Format (up to 25 µg/prep.)	50 preps.	R2024
ZR Tissue & Insect RNA MicroPrep™	Spin Column Format (up to 5 µg/prep.)	50 preps.	R2030
YeaStar RNA Kit™	Spin Column Format (up to 25 µg/prep.)	50 preps.	R1002
<b>RNA Clean-up, Concentration &amp; Recovery</b>			
RNA Clean & Concentrator™-5	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	R1015 R1016
RNA Clean & Concentrator™-25	Spin Column Format (up to 25 µg/prep.)	50 preps. 100 preps.	R1017 R1018
ZR-96 RNA Clean & Concentrator™	96-Well Format (up to 25 µg/well)	2x96 preps.	R1080
DNA-Free RNA Kit™	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	R1013 R1014
Zymoclean™ Gel RNA Recovery Kit	Spin Column Format (up to 5 µg/prep.)	50 preps.	R1011
ZR small-RNA™ PAGE Recovery Kit	Spin Column Format (up to 5 µg/prep.)	20 preps.	R1070
<b>small RNA Isolation &amp; Recovery</b>			
RNA Clean & Concentrator™-5	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	R1015 R1016
RNA Clean & Concentrator™-25	Spin Column Format (up to 25 µg/prep.)	50 preps. 100 preps.	R1017 R1018
ZR RNA MicroPrep™	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	R1060 R1061
ZR RNA MiniPrep™	Spin Column Format (up to 25 µg/prep.)	50 preps. 200 preps.	R1064 R1065
ZR small-RNA™ PAGE Recovery Kit	Spin Column Format (up to 5 µg/prep.)	20 preps.	R1070

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Toll Free: 1-888-882-9682 • Fax: 1-714-288-9643 • Web: [www.zymoresearch.com](http://www.zymoresearch.com) • E-mail: [info@zymoresearch.com](mailto:info@zymoresearch.com)