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 (≥ 6 µl).
- 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

RNA Clean & Concentrator™-5 (Kit Size)	**R1015** (50 Preps.)	**R1016** (200 Preps.)	**Storage Temperature**
RNA Binding Buffer	25 ml	100 ml	Room Temp.
RNA Prep Buffer	25 ml	4x 25 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	12 ml	2x 24 ml	Room Temp.
DNase/RNase-Free Water	1 ml	6 ml	Room Temp.
Zymo-Spin™ IC Columns	50	200	Room Temp.
Collection Tubes	50	200	Room Temp.
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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] 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

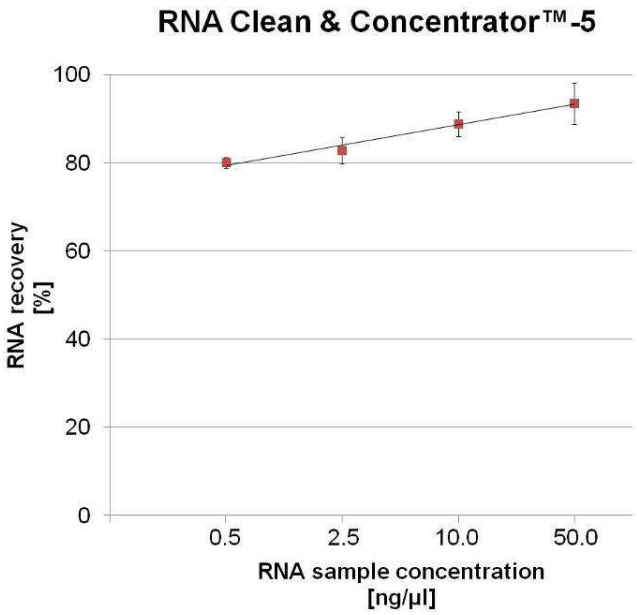
- **Sample Sources** – RNA fragments (≥17 nucleotides): DNase treated RNA, *in vitro* transcription products, the aqueous phase following an *acid phenol* extraction methods (*e.g.,* TRI Reagent®) *etc*.
- **Format** – Spin column.
- **RNA Purity** – High quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230}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

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.

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[1] *total RNA (>17nt)*	***OR***	**small RNA elimination**[2] *total RNA (>200nt)*
1.	Add 2 volumes of **RNA Binding Buffer** to each volume of RNA sample[3] and mix well.		Mix 1 volume of **RNA Binding Buffer** with 1 volume ethanol (95-100%).
2.	Add 1 volume ethanol (95-100%) to the mixture from *Step 1 (e.g., 200 µl ethanol and 200 µl mixture)*, and mix well.		Add 2 volumes of the adjusted buffer from *Step 1* to 1 volume of an RNA sample[3] *(e.g., 200 µl mixture and 100 µl RNA)* and mix well.

3. Transfer the mixture from *Step 2* to the **Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge at ≥12,000 x *g* for 1 minute[4]. Discard the flow-through.
4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge at ≥12,000 x *g* for 1 minute. Discard the flow-through.
5. Add 800 µl **RNA Wash Buffer** to the column and centrifuge at ≥12,000 x *g* for 30 seconds. Discard the flow-through. Repeat the wash step with 400 µl **RNA Wash Buffer**.
6. Centrifuge the **Zymo-Spin™ IC Column** in an emptied **Collection Tube** at ≥12,000 x *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 ≥6 µl of **DNase/RNase-Free Water**[5] directly to the column matrix and let stand for 1 minute at room temperature. Centrifuge at 10,000 *x g* for 30 seconds. The eluted RNA can be used immediately or stored at -70°C.

Notes:

[1] RNA species ≥17 nt will be recovered.

[2] RNA species ≤200 nt will be removed.

[3] Minimal recommended sample volume is 50 µl. Adjust samples <50 µl with RNase-free water.

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

[5] 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.

Ordering Information

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

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

2. Following *Step 3* of the RNA isolation protocol[1], 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 25-37ºC for ≥15 minutes[2], then centrifuge ≥12,000 x *g* for 30 seconds. Discard the flow-through.

5. Continue with *Step 4* of the RNA isolation protocol[3].

Notes:

[1] See page 3, Protocol – *Step 3*.

[2] The temperature optimum for DNase I activity is at 37 ºC. An optimal incubation time may vary.

[3] 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
DNase/RNase-Free Water	24 µl

2. Following *Step* 3 in the RNA isolation protocol[1], 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[2], 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[3].

Notes:

[1] See page 3, *Step 3*.

[2] The temperature optimum for DNase I activity is at 37 ºC. An optimal incubation time may vary.

[3] See page 3, *Step 4*.

RNA Purification Products - Zymo Research

Product	Description	Kit Size	Cat. No.
	Total RNA Isolation		
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**
	RNA Clean-up, Concentration & Recovery		
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**
	small RNA Isolation & Recovery		
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**