ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-96 RNA Clean & Concentrator™

Catalog No. **R1080**

Highlights

- Quick *(20 minute)* high-throughput (96-well) method for cleaning and concentrating RNA.
- *Fast-Spin* plate technology allows RNA to be eluted into minimal volumes (≥10 µl).
- Eluted RNA is ultra clean and ready for subsequent analysis and molecular manipulation.

Contents

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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

ZR-96 RNA Clean & Concentrator™ (Kit Size)	**R1080** (2x 96 Preps.)	**Storage Temperature**
RNA Binding Buffer	100 ml	Room Temp.
RNA Prep Buffer	2x 25 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	24 ml	Room Temp.
DNase/RNase-Free Water	4 ml	Room Temp.
Zymo-Spin™ I-96 Plate	2	Room Temp.
Collection Plate	2	Room Temp.
Elution Plate	2	Room Temp.
Cover Foil	4	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 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate before use.

Specifications

- **Sample Sources** – RNA fragments (≥17 nucleotides); e.g., DNase treated RNA, *in vitro* transcription products.
- **Format** – 96-well Plate
- **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 ≥10 µl RNase-free water allowing for a highly concentrated sample. The RNA binding capacity of the supplied **Zymo-Spin™ I-96 Plate** is ~25 µg/well.
- **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

Note:

For DNA clean-up see the **ZR-96 DNA Clean & Concentrator™-5** (Catalog Nos. D4023, D4024).

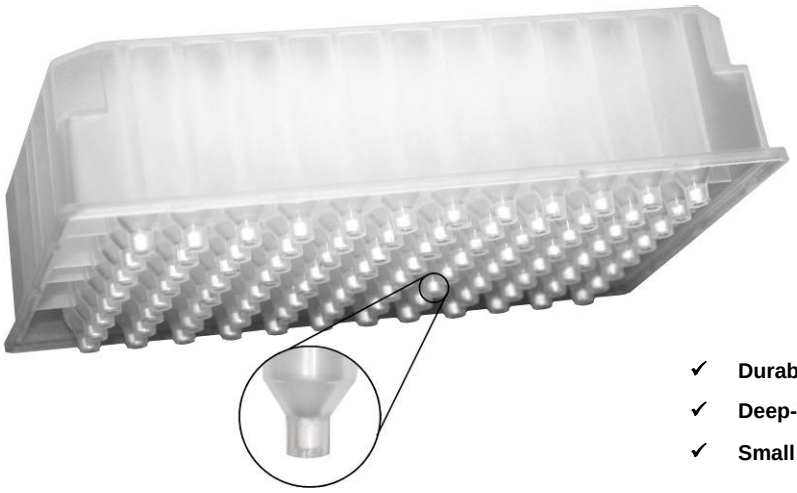
Product Description

The **ZR-96 RNA Clean & Concentrator™** provides a simple and reliable method for large-scale (96-well) purification and concentration of up to ~25 µg (per well) of high-quality RT-PCR-ready RNA. The procedure is based on the use of a unique single-buffer system and *Fast-Spin* plate technology.

The procedure features a single-buffer system that allows for efficient RNA adsorption from up to 96 samples onto the matrices of the supplied **Zymo-Spin™ I-96 Plate**. The RNA is washed twice then eluted and concentrated into ≥10 µl of RNase-free water.

The kit allows for small RNA recovery or removal by alternating the first two steps of the purification procedure. 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 RNA isolation procedure typically takes about 20 minutes.



- ✓ **Durable Construction**
- ✓ **Deep-Well Design**
- ✓ **Small Elution Volume**

The **Zymo-Spin™ I-96 Plate** design.

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

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

Buffer Preparation

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

Protocol

Follow part A or B of the protocol before proceeding to Step 3.

A. To recover total RNA including small RNAs[1]:

1a. Mix 1 volume of an RNA sample with 2 volumes of the **RNA Binding Buffer** *(e.g., 100 µl RNA sample and 200 µl buffer)*[2] by first aliquoting the **RNA Binding Buffer** to the wells of the **Zymo-Spin™ I-96 Plate** mounted on a **Collection Plate** and then adding RNA samples. Mix well[3,4].

2a. Add 1 volume 95-100% ethanol to the mixture from Step 1a *(e.g., 300 µl ethanol and 300 µl mixture)*, mix well and proceed to Step 3 below.

B. To remove the small RNA species from an RNA sample[5]:

1b. Prepare a 0.5x **RNA Binding Buffer** by mixing 1 volume of **RNA Binding Buffer** with 1 volume ethanol (95-100%).

2b. Mix 1 volume of an RNA sample with 2 volumes of the 0.5× **RNA Binding Buffer** from Step 1b *(e.g., 100 µl RNA sample and 200 µl buffer)*[2] by first aliquoting the 0.5× **RNA Binding Buffer** to the wells of the **Zymo-Spin™ I-96 Plate** mounted on a **Collection Plate** and then adding RNA samples[3,4].

3. Centrifuge the **Zymo-Spin™ I-96 Plate** from Step 2a or 2b, mounted on a **Collection Plate**, at 5,000 x *g* for 5 minutes[6]. Discard the flow-through and mount the **Zymo-Spin™ I-96 Plate** back onto the **Collection Plate**.

4. Add 400 µl **RNA Prep Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge at 5,000 x *g* for 5 minutes. Discard the flow-through.

5. Add 800 µl **RNA Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge at 5,000 x *g* for 2 minutes. Discard the flow-through. Repeat the wash step with 400 µl **RNA Wash Buffer** and centrifuge at 5,000 x *g* for 2 minutes. Discard the flow-through.

6. Centrifuge the **Zymo-Spin™ I-96 Plate** at 5,000 x *g* for 5 minutes in the emptied **Collection Plate** to ensure complete removal of the wash buffer.

7. Transfer the **Zymo-Spin™ I-96 Plate** onto the **Elution Plate**. Add ≥10 µl **RNase-Free Water** directly to the column matrix in each well[7] and let stand for 1 minute at room temperature. To elute the RNA, centrifuge at 5,000 x *g* for 5 minutes. RNA can be used immediately or stored[8] at ≤-70 ºC.

Notes:

[1] RNA species ≥17 nt (e.g., miRNAs) will be recovered.

[2] Add 100 µl **RNA Binding Buffer** (Step 1a) or 0.5× **RNA Binding Buffer** (Step 1b) to all samples ≤50 µl.

[3] *Example:* Mix by pipetting or vortex a sealed 96-well plate. **Collection Plate** may be also used for the sample preparation. (If a **Collection Plate** is used for mixing, use the **Cover Foil** and quick spin prior to the next step.)

[4] The sample capacity of each well of the **Collection Plate** is ~800 µl. It may be necessary to load and spin the **Zymo-Spin™ I-96 Plate** multiple times to process samples >800 µl.

[5] Small RNA species ≤200 nt will be removed

[6] **Optional DNase treatment:** Following Step 3, samples can be in-column DNase treated. See **Appendix** (page 5).

[7] For quantitative recovery of RNA samples, increase the elution volume (>15 µl) and/or repeat the elution Step 7.

[8] To prevent an evaporation and to store purified RNA samples on the **Elution Plate** use the **Cover Foil**.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR-96 RNA Clean & Concentrator™	R1080	2x 96 Preps.

For Individual Sale	Catalog No.	Amount
RNA Binding Buffer	R1013-2-50 R1013-2-100	50 ml 100 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
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
Zymo-Spin™ I-96 Plates	C2004	2 plates
Collection Plates	C2002	2 plates
Elution Plates	C2003	2 plates

Fast-Spin plate technology employed in the 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

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/well 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™ I-96 Plate** mounted on a **Collection Plate** and centrifuge at 5,000 x *g* for 5 minutes. Discard the flow through.

3. Add 30 µl DNase I cocktail from Step 1 above directly to the matrix of the **Zymo-Spin™ I-96 Plate**. Keep the **Zymo-Spin™ I-96 Plate** mounted on a **Collection Plate**.

4. Incubate the plate at 25-37ºC for ≥15 minutes[2], then centrifuge at 5,000 x *g* for 5 minutes.

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.

Related Products

Product	Description	Kit Size	Cat No.
	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. 200 Preps.	**R1017** **R1018**
ZR-96 RNA Clean & Concentrator™	96-Well Format (up to25 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**