ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-96 RNA Clean & Concentrator™

Catalog Nos. **R1080**

Highlights

- High throughput (96-well) method for recovery of ultra pure RNA (≥17 nt) from enzymatic reactions, aqueous phase (following Trizol® extraction) and other sources.
- High-quality RNA eluted in ≥10 µl is ready for reverse transcription, microarray, sequencing, etc.

Contents

ZYMO RESEARCH CORP.

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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.

Notes:

[1] Before use, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1080).

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

Product Contents

ZR-96 RNA Clean & Concentrator™ (Kit Size)	**R1080** (2x 96 Preps.)
RNA Binding Buffer	100 ml
RNA Prep Buffer	4x 25 ml
RNA Wash Buffer[1] (concentrate)	2x 24 ml
DNase/RNase-Free Water	6 ml
Zymo-Spin™ I-96 Plate	2
Collection Plate	2
Elution Plate	2
Cover Foil	4
Instruction Manual	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.

Storage Temperature – Store all kit components (*i.e.,* buffers, columns) at room temperature.

Specifications

- **Sample Sources** – DNase I treated RNA, *in vitro* transcription products, the aqueous phase following TRIzol®/chloroform or similar[2] extraction (page 4).
- **RNA Size Limits** – From 17 nt to ~23 kb.
- **Format** – 96-well Plate
- **RNA Purity** – High quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) suitable for reverse transcription, microarray, sequencing etc.
- **RNA Recovery** – Up to ~25 µg RNA/well can be eluted into as little as ≥10 µl RNase-free water allowing for a highly concentrated sample.
- **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** – Centrifuge with 96-well plate carrier.

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®, TRIzol® and RNAzol® (Molecular Research Center, Inc.), QIAzol® (Qiagen GmbH), TriPure™ (Roche Diagnostics Operations, Inc.), TriSure™ (Bioline Ltd.), RNA*later*® (Ambion, Inc.).

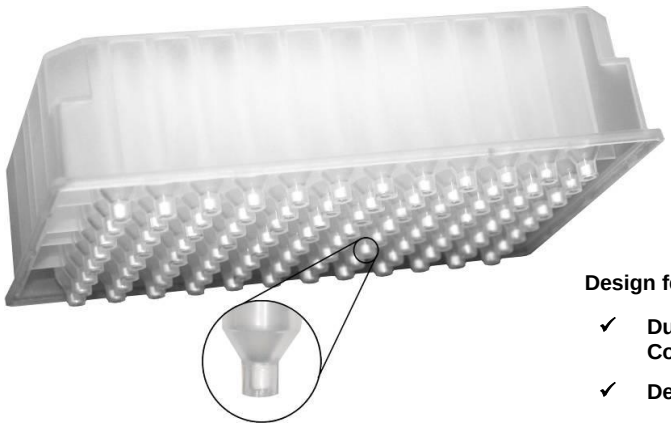
Product Description

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

The kit allows for efficient RNA clean-up from up to 96 samples with the supplied **Zymo-Spin™ I-96 Plate**. The RNA is washed twice then eluted and concentrated into ≥10 µl of DNase/RNase-free water. Protocol for DNase I digestion is provided.

RNA ≥17 nucleotides 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 30 minutes.



Zymo-Spin™ I-96

Design features:

- ✓ **Durable Construction**
- ✓ **Deep-Well**

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 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1080).

Protocol

All centrifugation steps should be performed at 3,000-5,000 *x g.*
RNA species ≥17 nt will be recovered.

1. Add 2 volumes **RNA Binding Buffer** to each sample and mix.

   Example: Mix 200 µl buffer and 100 µl sample.

2. Add an equal volume of ethanol (95-100%) and mix.

   Example: Add 300 µl ethanol.

3. Transfer the sample to each well of a **Zymo-Spin™ I-96 Plate**[1] mounted on a **Collection Plate** and centrifuge for 5 minutes[2]. Discard the flow-through.

4. Add 400 µl/well **RNA Prep Buffer** and centrifuge for 5 minutes. Discard the flow-through.

5. Add 700 µl/well **RNA Wash Buffer** and centrifuge for 5 minutes. Discard the flow-through.

6. Add 400 µl/well **RNA Wash Buffer** and centrifuge for 5 minutes to ensure complete removal of the wash buffer. Mount the plate carefully onto an **Elution Plate**.

7. Add ≥10 µl/well **DNase/RNase-Free Water** directly to the matrix and centrifuge for 5 minutes.

   The eluted RNA can be used immediately or stored frozen. Use the **Cover Foil** to prevent evaporation.

Notes:

[1] To process samples >800 µl, **Zymo-Spin™ I-96 Plate** may be reloaded.

[2] At this point, samples can be in-column DNase treated (page 4).

DNase I treatment

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)[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 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 (page 3, step 1).

(II) In-column

All centrifugation steps should be performed at 3,000-5,000 *x g*.

1. Following the RNA binding step (page 3, step 3), add 400 µl/well **RNA Wash Buffer**. Centrifuge for 5 minutes. 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 **DNase I reaction mix** directly to the matrix. Incubate at room temperature (20-30ºC) for 15 minutes. Then continue with RNA purification (page 3, step 4).

RNA purification from aqueous phase after TRIzol® extraction

1. Following Trizol®/chloroform or similar[2] extraction, carefully transfer the upper aqueous phase into an RNase-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. Then continue with purification (page 3, step 3).

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.

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

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	R1013-2-25 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 R1060-2-100	10 ml 25 ml 100 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™ I-96 Plates	C2004	2 plates
Collection Plates	C2002	2 plates
Elution Plates	C2003	2 plates
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml

DNA PURIFICATION

What is Clean-Spin™ Technology?

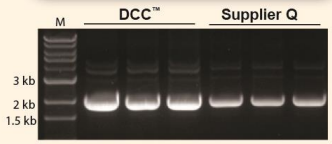
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small (≥6 µl) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

Product	Size (Cat. No.)
DNA Clean & Concentrator™-5	50 Preps. (D4013) 200 Preps. (D4014)
ZR-96 DNA Clean & Concentrator™-5	2 x 96 Preps. (D4023) 4 x 96 Preps. (D4024)
Genomic DNA Clean & Concentrator™	25 Preps. (D4010) 100 Preps. (D4011)



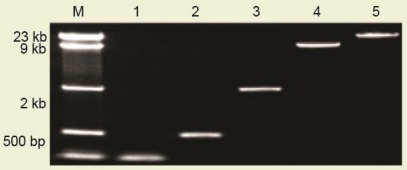
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥6 µl.
- ✓ Ultra-pure DNA ideal for DNA ligation, sequencing, etc.
- ✓ Format also available for large DNA >20 kb.

Product	Size (Cat. No.)
Zymoclean™ Gel DNA Recovery Kit	50 Preps. (D4001) 200 Preps. (D4002)
Zymoclean™ Large Fragment DNA Recovery Kit	25 Preps. (D4045) 100 Preps. (D4046)



DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

Recover transfection-quality plasmid DNA directly from culture

Zyppy™ Plasmid Prep Kits

- ✓ The fastest, simplest method available for purifying high quality plasmid DNA from *E. coli.*
- ✓ Pellet-Free™ procedure omits conventional cell-pelleting and resuspension steps.
- ✓ Transfection quality plasmid DNA directly from culture in under 15 minutes.

Easy, Pellet-free Procedure: Add Lysis Buffer ***Directly*** to Bacterial Culture

Product	Size (Cat. No.)
Zyppy™ Plasmid Miniprep Kit	50 Preps. (D4036) 100 Preps. (D4019) 400 Preps. (D4020) 800 Preps. (D4037)

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RNA PURIFICATION

What is Clean-Spin™ Technology?

The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

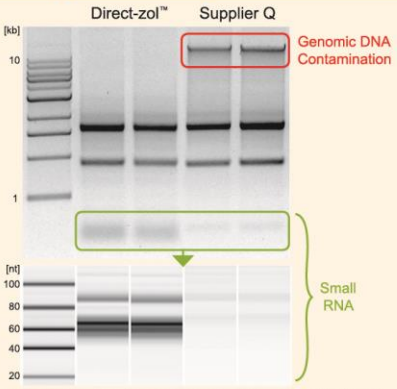
Get RNA *directly* from TRIzol® without phase separation

Direct-zol™ RNA

- ✓ For purification of high-quality small and large RNA *directly* from TRIzol®, TRI Reagent®, or similar.
- ✓ Bypasses phase separation and precipitation procedures allowing for unbiased recovery of miRNA

Product	Size (Cat. No.)
Direct-zol™ RNA MiniPrep	50 Preps. (R2050) 50 Preps. (R2051)* 200 Preps. (R2052) 200 Preps. (R2053)*
96-well and MagBead formats also available!	

DNase I included in all kits.
* Supplied with *TRI-Reagent®*



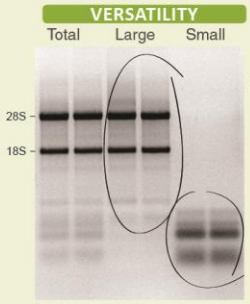
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is ***DNA-free***.

Isolate DNA-free RNA from 1 to 10^7 cells in minutes

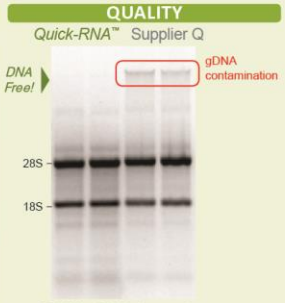
Quick-RNA™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to 10^7 cells.
- ✓ DNA-free RNA ideal for any downstream application – *DNase I included.*

Product	Size (Cat. No.)
Quick-RNA™ MicroPrep	50 Preps. (R1050) 200 Preps. (R1051)
Quick-RNA™ MiniPrep	50 Preps. (R1054) 200 Preps. (R1055)
ZR-96 *Quick-RNA*™	2 x 96 Preps. (R1052) 4 x 96 Preps. (R1053)



Isolate total, large, or small RNA with the *Quick-RNA*™ kit.



RNA is DNA-free using the *Quick-RNA*™ kit

Purify RNA from enzymatic and labeling reactions in 5 minutes

RNA Clean & Concentrator™

- ✓ Recover ultra-pure RNA in small (≥6 µl) elution volumes.
- ✓ Compatible with TRIzol®, phenol, choloform, and RNase inhibitors (RNAlater®).
- ✓ RNA is ideal for RT-PCR, q-PCR, hybridization, arrays, RNA interference, etc.

Product	Size (Cat. No.)
RNA Clean & Concentrator™-5	50 Preps. (R1015) 200 Preps. (R1016)
RNA Clean & Concentrator™-25	50 Preps. (R1017) 100 Preps. (R1018)
ZR-96 RNA Clean & Concentrator™	2x96 well plates (R1080)
DNA-Free RNA Kit™	50 Preps. (R1013) 200 Preps. (R1014)

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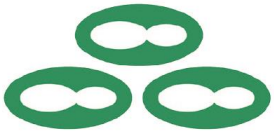

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