



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

DNA-Free RNA Kit™

Catalog Nos. (R1013, R1014, R1027, & R1028)

Contents

I. General Information.....	1 - 3
Description	
Highlights	
Specifications	
Product Contents	
Ordering Information	
II. Protocol.....	3, 4
Troubleshooting	
III. List of Products.....	5

For Laboratory Use Only

ZYMO RESEARCH CORP.

Toll Free: 1-888-882-9682 • Fax: 1-714-288-9643 • Web: www.zymoresearch.com • E-mail: info@zymoresearch.com

I. General Information

Description

Zymo Research's **DNA-Free RNA Kit™** provides a hassle-free method for the rapid preparation of DNA-free, high quality RNA for manipulations such as RT-PCR. The procedure includes the use of certified RNase-free DNase followed by Fast-Spin column technology to yield purified RNA in small volumes of RNase-free water in just minutes. The result is highly-concentrated, DNA-free RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, etc.

Highlights

- Convenient method for DNA-free RNA preparation.
- Column design allows RNA to be eluted at high concentrations into minimal volumes of RNase-free water in just minutes.
- Purified RNA is suitable for use in RNA-based procedures including RT-PCR.
- Omits the use of organic denaturants.

Specifications

- **RNA Purity** – High quality, purified RNA is eluted into RNase-free water and is suitable for RNA-based manipulations including RT-PCR.
- **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 column is 5 µg.
- **RNA Storage** – Recommended that 1 U/10 µl RNase inhibitor be added to the RNA prior to storage at -70°C.
- **Sample Sources** – RNA preparations containing DNA.
- **Stability of Product Reagents** – Integrity of kit components is guaranteed for up to one year from date of purchase.
- **Quality Control** – Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

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

Product Contents

DNA-Free RNA Kit™ Kit Size	R1013, R1027 (50 Preps.)	R1014, R1028 (200 Preps.)	Storage Temperature
RNA Binding Buffer	50 ml	2x100 ml	Room Temp.
RNA Wash Buffer*	6 ml	24 ml	Room Temp.
DNase I (100 U/μl) / 10X DNase I Buffer	1 set	4x1 set	-20°C
DNase/RNase-Free Water	1 ml	2x1 ml	Room Temp.
Zymo-Spin™ Columns	50 ct.	4x50 ct.	Room Temp.
Collection Tubes	50 ct.	4x50 ct.	Room Temp.
Instruction Manual	1	1	-

* Ethanol must be added prior to use as indicated on **RNA Wash Buffer** label.

Ordering Information

Product Description	Catalog No.	Kit Size
DNA-Free RNA Kit™ supplied w/ capped columns	R1013	50 preps.
DNA-Free RNA Kit™ supplied w/ uncapped columns	R1027	50 preps.
DNA-Free RNA Kit™ supplied w/ capped columns	R1014	200 preps.
DNA-Free RNA Kit™ supplied w/ uncapped columns	R1028	200 preps.

For Individual Sale	Catalog No.	Amount
DNase I (100 U/μl) / 10X DNase I Buffer	E1007	1 set
RNA Binding Buffer	R1013-2-50	50 ml
	R1013-2-100	100 ml
RNA Wash Buffer (concentrate)	R1003-3-2.4	2.4 ml
	R1003-3-6	6 ml
	R1003-3-12	12 ml
	R1003-3-24	24 ml
	R1003-3-48	48 ml
DNase/RNase Free Water	W1001-1	1ml
	W1001-4	4 ml
	W1001-6	6 ml
	W1001-10	10 ml

ZYMO RESEARCH CORP.

Toll Free: 1-888-882-9682 • Fax: 1-714-288-9643 • Web: www.zymoresearch.com • E-mail: info@zymoresearch.com

For Individual Sale Cont.	Catalog No.	Amount
Zymo-Spin I™ Columns (uncapped)	C1003-50	50 ct.
	C1003-250	250 ct.
Zymo-Spin IC™ Columns (capped)	C1004-50	50 ct.
	C1004-250	250 ct.
Collection Tubes	C1001-50	50 ct.
	C1001-500	500 ct.
	C1001-1000	1000 ct.

II. Protocol

Overview

The **DNA-Free RNA Kit™** allows for removal of DNA from RNA preparations via DNase I treatment followed by adsorption of the RNA onto the matrix of a **Zymo-Spin™ Column**. The RNA is washed twice then eluted with a minimal volume of RNase-free water. The entire procedure typically takes 15-20 minutes.

Reagent Preparation

- Before starting, add 24 ml 100% ethanol to the 6 ml **RNA Wash Buffer** concentrate (96 ml 100% ethanol to the 24 ml wash buffer concentrate) to obtain the final **RNA Wash Buffer** solution.
- Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

For **Technical Assistance** contact those at **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

Note: Alternatively, add 26 ml and 104 ml of 95% ethanol to the 6 ml and 24 ml sizes of the wash buffer concentrate, respectively.

Method

1. Digest RNA samples with **DNase I** in a 10-100 μ l final reaction volume. Example: For a 50 μ l final reaction volume setup is as follows...

20 μ l RNA Sample ($\leq 5 \mu$ g) in Water or TE Buffer
 5 μ l **10X DNase I Buffer**
 2.5 μ l RNase-Free **DNase I**
22.5 μ l RNase-Free Water
 50 μ l Final Reaction Volume

Mix and incubate at 37°C for 10-15 minutes.

2. Add 4 volumes of **RNA Binding Buffer**, mix.
3. Transfer the mixture to a **Zymo-Spin™ Column** in a collection tube.
4. Centrifuge at $\geq 10,000$ rpm for 30 seconds. Discard the flow-through.
5. Add 200 μ l of **RNA Wash Buffer** to the column. Centrifuge at $\geq 10,000$ rpm for 30-60 seconds. Discard flow-through. Repeat wash step.
6. Add 8-10 μ l of **DNase/RNase-free Water** directly to the column matrix. Place column in an RNase-free 1.5 ml tube. Centrifuge at $\geq 10,000$ rpm for 30-60 seconds to elute the RNA.

The eluted RNA can be used immediately or stored at -70°C for future use.

Troubleshooting

1. DNase I activity depends on the presence of Mg^{2+} ions, thus high concentrations of EDTA present in the RNA sample may interfere with the DNase I digestion. The final Mg^{2+} concentration in the DNase I digestion reaction is 5 mM. It is important to make sure that the Mg^{2+} concentration remains above 2 mM if the RNA sample should contain EDTA.
2. RNA samples from some virus-infected cells may contain viral DNA/RNA hybrids. Such hybrids may be refractory to DNase I treatment.

Note: Water is strongly recommended to elute the RNA. 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 one minute prior to eluting the RNA may increase RNA yield. Also, the yield may be increased by performing a second elution with another 6-8 μ l of water and pooling it with the first.

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 chaotropic and are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Popular RNA Purification & Isolation Products from Zymo

Product	Description	Kit Size	Cat No. (Column Format)
Pinpoint Slide RNA Isolation System I™	Isolation of total RNA from targeted tissue areas on a microscope slide of fresh or frozen tissue sections. Perfect for RNA isolation from clinical tissue samples.	50 Preps	R1003
Pinpoint Slide RNA Isolation System II™	Isolation of total RNA from targeted tissue areas on a microscope slide of paraffin embedded tissue sections. Perfect for RNA isolation from clinical tissue samples.	50 Preps	R1007
Mini RNA Isolation I Kit™	Isolation of trace amounts of RNA from 1×10^1 to 1×10^5 cells. Available in both capped and uncapped column formats.	50 Preps 50 Preps	R1005 (Uncapped) R1006 (Capped)
Mini RNA Isolation II Kit™	Isolation of RNA from 10^2 to 5×10^6 cells. Available in both capped and uncapped column formats.	50 Preps 200 Preps 50 Preps 200 Preps	R1030 (Capped) R1031 (Capped) R1032 (Uncapped) R1033 (Uncapped)
ZR Whole Blood Total RNA Kit™	Isolation of RNA from whole blood samples in 15 minutes.	50 Preps 100 Preps	R1020 R1021
ZR Viral RNA Kit™	Isolation of viral RNA from cell-free body fluids or sample mixtures containing cells at a concentration less than 10^5 cells per ml.	50 Preps 200 Preps	R1034 R1035
ZR-96 Viral RNA Kit™	High-output isolation of viral RNA from cell-free body fluids or sample mixtures containing cells at a concentration less than 10^5 cells per ml.	2x96 Preps 4x96 Preps	R1040 R1041
Urine RNA Kit™	Isolation of total RNA from urine sediment samples. The system employs a unique urine filter to collect cells via a simple syringe "push-through" method.	20 Preps 50 Preps	R1038 R1039
RNA Clean-up Kit-5™	Clean and concentrate 5 µg RNA from any reaction in 2 minutes. 8 µl minimal elution volume. Available in capped and uncapped column format.	50 Preps 200 Preps 50 Preps 200 Preps	R1015 (Capped) R1016 (Capped) R1023 (Uncapped) R1024 (Uncapped)
RNA Clean-up Kit-25™	Clean and concentrate up to 25 µg RNA from any reaction in 2 minutes. 50 µl minimal elution volume. Available in capped and uncapped column format.	50 Preps 200 Preps 50 Preps 200 Preps	R1017 (Capped) R1018 (Capped) R1025 (Uncapped) R1026 (Uncapped)
DNA Free RNA Kit™	Efficiently removes DNA from RNA preparations for RT-PCR reactions in 15 minutes. Available in capped and uncapped column format.	50 Preps 200 Preps 50 Preps 200 Preps	R1013 (Capped) R1014 (Capped) R1027 (Uncapped) R1028 (Uncapped)
Zymoclean Gel RNA Recovery Kit™	Isolation of RNA from agarose gels in 15 minutes. 8 µl minimal elution volume for maximum concentration of RNA.	50 Preps	R1011
YeaStar RNA Kit™	Isolation of RNA from a broad spectrum of fungi susceptible to yeast lytic enzyme lysis.	40 Preps	R1001