



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Whole-Blood Total RNA Kit™

Catalog Nos. (R1020 & R1021)

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For Laboratory Use Only

ZYMO RESEARCH CORP.

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I. General Information

Description

Zymo Research's **ZR Whole-Blood Total RNA Kit™** provides a hassle-free method for the rapid isolation of total RNA from whole-blood in as little as 10 minutes. This simple procedure is based on the use of a unique single-buffer system combined with Fast-Spin column technology. The procedure is easy: just add the buffer to a blood sample and the extracted RNA is then isolated in RNase-free water using a **Zymo-Spin IIIC™ Column**. Optionally, the RNA can be further purified and concentrated into 6-10 µl of RNase-free water using a **Zymo-Spin IC™ Column**. RNA can be isolated immediately from fresh samples or at a later time from blood stored in **ZR RNA Buffer™**. This product is designed to purify and concentrate RNA from blood for subsequent RNA-based methods including RT-PCR, hybridization, etc.

Highlights

- Convenient method for purifying total RNA from whole-blood samples.
- Compatible with commonly used anticoagulants (i.e., EDTA, heparin, citrate).
- 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 and proteinases.

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 40 µl RNase-free water or (optionally) 6-10 µl for a highly concentrated sample. The RNA binding capacity of the **Zymo-Spin IC™ 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** – Whole-blood (fresh or stored in **ZR RNA Buffer™**).
- **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

ZR Whole-Blood Total RNA Kit™ Kit Size	R1020 (50 Preps.)	R1021 (100 Preps.)	Storage Temperature
ZR RNA Buffer™	50 ml	100 ml	Room Temp.
RNA Pre-Wash Buffer	25 ml	50 ml	Room Temp.
RNA Wash Buffer*	24 ml	24 ml	Room Temp.
DNase/RNase-Free Water	6 ml	2x6 ml	Room Temp.
Zymo-Spin IC™ Columns	50 ct.	2x50 ct.	Room Temp.
Zymo-Spin IIIC™ Columns	50 ct.	2x50 ct.	Room Temp.
Collection Tubes	3x50 ct.	6x50 ct.	Room Temp.
RNase-Free Tubes	2x50 ct.	-	Room Temp.
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* Ethanol must be added prior to use as indicated on **RNA Wash Buffer** label.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR Whole-Blood Total RNA Kit™	R1020	50 preps.
ZR Whole-Blood Total RNA Kit™	R1021	100 preps.

For Individual Sale	Catalog No.	Amount
ZR RNA Buffer™	R1020-1-50	50 ml
	R1020-1-100	100 ml
RNA Pre-Wash Buffer	R1020-2-25	25 ml
	R1020-2-50	50 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
DNase/RNase-Free Water	R1003-3-48	48 ml
	W1001-1	1 ml
	W1001-4	4 ml
	W1001-6	6 ml
Zymo-Spin IC™ Columns	W1001-10	10 ml
	C1004-50	50 ct.
Zymo-Spin IIIC™ Columns	C1004-250	250 ct.
	C1006-50	50 ct.
	C1006-250	250 ct.

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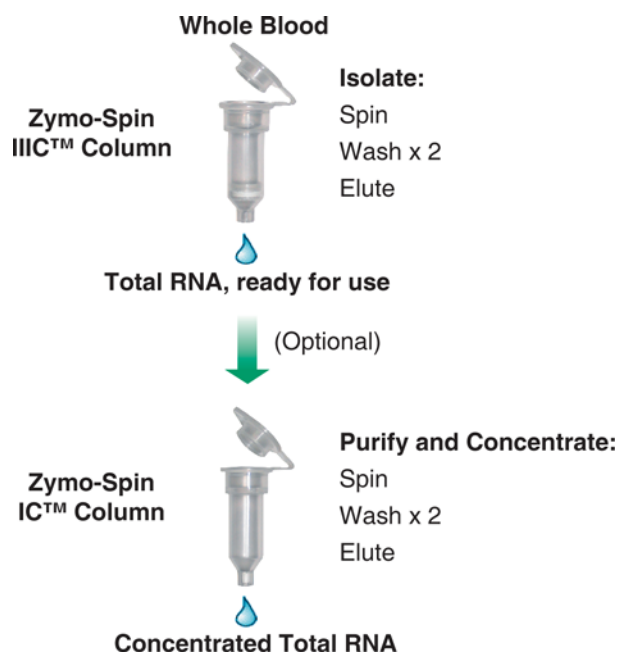
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For Individual Sale Cont.	Catalog No.	Amount
Collection Tubes	C1001-50	50 ct.
	C1001-500	500 ct.
	C1001-1000	1,000 ct.
RNase-Free Tubes	C2001-50	50 ct.

II. Protocol

Overview

The **ZR Whole-Blood Total RNA Kit™** is a convenient method for purifying RNA from fresh or stored whole-blood samples in **ZR RNA Buffer™** using Zymo Research's Fast-Spin column technology. As shown below, RNA is isolated using a **Zymo-Spin IIIC™ Column** and then (optionally) concentrated using a **Zymo-Spin IC™ Column**. In the latter step, the RNA is washed twice then eluted with a minimal volume (8-10 µl) of RNase-free water. The entire procedure typically takes about 10-15 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: Immediate processing of blood samples is recommended. Should storage of blood be required prior to processing, see Note 2 on Page 5.

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

The following protocol is based on a 200 μ l sample volume of blood.

1. Add 600 μ l **ZR RNA Buffer™** to 200 μ l whole-blood sample and mix.
2. Transfer the mixture to a **Zymo-spin IIIC™ Column** in a **Collection Tube**.
3. Centrifuge $\geq 10,000$ rpm (10,000-12,000 x g) for 30-60 seconds. Discard the **Collection Tube** containing the flow-through.
4. Add 400 μ l of **RNA Prewash Buffer** to the column. Centrifuge at $\geq 10,000$ rpm for 30-60 seconds. Discard flow-through.
5. Add 400 μ l of **RNA Wash Buffer** to the column. Centrifuge at $\geq 10,000$ rpm for 30-60 seconds. Discard flow-through.
6. Add 50 μ l of **DNase/RNase-Free Water** directly to the column matrix. Place column into an **RNase-free 1.5 ml tube**. Centrifuge briefly to elute the RNA.

The RNA is ready for use at this time; however, if the RNA requires concentration, follow the steps below.

(optional)

1. Add 400 μ l of **ZR RNA Buffer™** to the eluted RNA and mix.
2. Transfer the mixture to a **Zymo-Spin IC™ Column** in a **Collection Tube**.
3. Centrifuge at $\geq 10,000$ rpm for 30-60 seconds. Discard the flow-through.
4. 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.
5. Add 8-10 μ l of **DNase/RNase-Free Water** directly to the column matrix. Place column into an **RNase-free 1.5 ml tube**. Centrifuge at $\geq 10,000$ rpm for 30-60 seconds to elute the RNA.

The purified RNA can be used immediately or stored at -70°C .

Note: The amount of blood used can be adjusted depending on the application. Should this be the case, adjust step 1 accordingly and follow the other steps as directed (See Note 1 on Page 5). If the blood sample can't be processed immediately, the blood can be "stabilized" for processing at a later time (see Note 2 on Page 5).

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.

Notes

1. **RNA Purification Using Different Volumes of Whole-Blood.** If RNA needs to be purified from different volumes of blood than that indicated in the standard Protocol (200 μ l), simply add 3 volumes of **ZR RNA Buffer** to every volume of whole blood used. (For 150 μ l blood, add 450 μ l of **ZR RNA Buffer™**). Adhere to the other steps as indicated in the protocol. One **Zymo-Spin IIC™ Column** can process up to 400 μ l blood through sequential loading of sample.
2. **Delayed Processing of Blood Samples:** Immediate processing of blood for RNA purification is highly recommended. However, if the blood cannot be processed immediately, the sample can be “stabilized” in **ZR RNA Buffer™** for RNA purification at a later time. To do this, simply add 3 volumes of **ZR RNA Buffer** to every volume of whole-blood. Blood samples mixed with **ZR RNA Buffer** can be stored at room temperature for 2 days, 0-4 °C for up to 5 days, -20 °C for up to 4 weeks, and -70 °C for over 6 months. After allowing a “stabilized” sample to reach room temperature, proceed with step 2 in the standard protocol.

Troubleshooting

1. **RNA Degradation:** Check for RNase contamination of buffers. All buffers and contents supplied by Zymo Research are certified RNase-free. RNases may be introduced during execution of the purification procedure. Exercise all necessary precautions to ensure the procedure is conducted in an RNase-free environment.
2. **Problems with RNA in Subsequent Experiments:** Make sure to add 3 volumes of **ZR RNA Buffer** to every volume of whole-blood. Also, make sure spin procedures are complete, as incomplete washing of salts and buffers may adversely affect the outcome of the procedure.
3. **DNA Contamination:** The combination of buffers and columns provided in this kit are optimized to efficiently isolate and purify RNA and exclude DNA. Additional DNA removal may be required if subsequent applications are affected by trace amounts of DNA. This can be accomplished using the **DNA-Free RNA kit™** (Cat. Nos. R1013 & R1014) from Zymo Research.

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