ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Whole Blood

RNA from any blood sample

Highlights

- Spin-column purification of total RNA (including small/microRNAs) from whole and partitioned blood samples. Compatible with commonly used anticoagulants (i.e., EDTA, citrate, heparin).
- DNA/RNA Shield™ and Proteinase K are included for unique worry-free sample storage at ambient temperatures and lysis technology, respectively.
- DNA-free RNA is ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:
R1201

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Quick-RNA™ Whole Blood	R1201 (50 prep)
DNA/RNA Shield™ (2X concentrate)	25 ml (x2)
RNA Recovery Buffer	10 ml
RNA Prep Buffer	25 ml
RNA Wash Buffer[1]	24 ml
DNase/RNase-Free Water	4 ml
DNase I[2] (lyophilized)	250 U
DNA Digestion Buffer	4 ml
Proteinase K[3] (lyophilized) & Storage Buffer	20 mg (x2)
PK Digestion Buffer	20 ml (x2)
Zymo-Spin™ IIICG Columns	50
Zymo-Spin™ IC Columns	50
Collection Tubes	100
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
2 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
#E1009-A (250 U), add 275 µl **water**
3 Add **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 20 mg, see Buffer Preparation, page 4. Store frozen aliquots.

Specifications

- **Sample Sources –** Up to 1 ml mammalian whole blood (fresh or stored in **DNA/RNA Shield™**), plasma, CSF or serum. Also compatible with pelleted blood cells (PBMCs, WBCs, buffy coat, PAXgene™ Blood RNA Tube cell pellet) and up to 50 µl nucleated blood.

- **Sample Preservation and Inactivation – DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents (e.g., virus, pathogens) and is ideal for safe sample storage and transport at ambient temperatures.

- **Size –** Total RNA including small/microRNAs (≥ 17 nt).

- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

- **Binding Capacity – Zymo-Spin™ IC Column** yield up to 10 µg RNA.

- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water.**

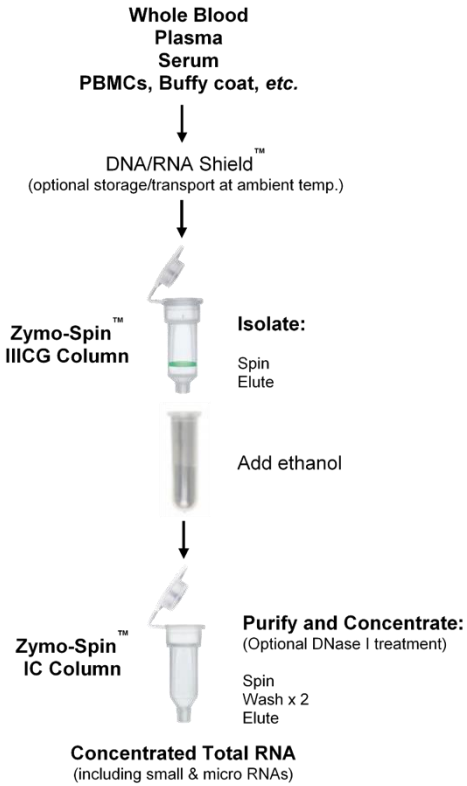
- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex, heat block, water bath or incubator.

- **Materials** (available separately) **–** RBC Lysis Buffer (R1022-2-50, R1022-2-100), for lysing red blood cells from fresh whole blood.

Product Description

The **Quick-RNA™ Whole Blood** kit utilizes **DNA/RNA Shield™**, a unique preservation and lysis technology, to enable rapid isolation of total RNA from whole or partitioned blood or a cell pellet (after red blood cell lysis).

The procedure uses Zymo-Spin™ column technology in which the sample is pre-filtered on the **Zymo-Spin™ IIICG Column,** then purified and concentrated on the **Zymo-Spin™ IC Column**. RNA is eluted into ≥ 6 µl of DNase/RNase-Free Water and is ready for any downstream application including RT-PCR, sequencing, etc.



Protocol

The protocol consists of: (I) Buffer Preparation and (II) RNA Purification.

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
  #E1009-A (250 U), add 275 µl **water**
- ✓ Add 1,040 µl **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 20 mg Vortex to dissolve and store frozen aliquots.
- ✓ For RNA purification from pelleted blood cells (page 6) or nucleated whole blood (page 7), prepare a 1X solution of **DNA/RNA Shield™** by adding an equal volume of nuclease-free water (not provided) to the **DNA/RNA Shield™** (2X concentrate) (1:1) and mix well.

(II) RNA Purification (whole blood)

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Add 200 µl **DNA/RNA Shield** (2X concentrate) to 200 µl whole blood[1] (1:1) and mix thoroughly.
2. Add 8 µl **Proteinase K** and mix. Incubate for 30 minutes.
3. Add an equal volume of isopropanol (1:1) and mix by vortexing.

   Example: Add 400 µl isopropanol to 400 µl mixture (**Proteinase K**-treated sample).
4. Transfer the mixture into a **Zymo-Spin™ IIICG Column**[2] (**green**) in a **Collection Tube** and centrifuge[3]. Then transfer the column into a nuclease-free tube (not provided).
5. Add 200 µl **RNA Recovery Buffer** to the column and centrifuge. Save the flow-through!
6. To the flow-through, add 200 µl ethanol (95-100%) and mix well.
7. Transfer the mixture into a **Zymo-Spin™ IC Column**[2] in a **Collection Tube** and centrifuge. Discard the flow-through.
8. **DNase I**[4] treatment (recommended)
   (D1) Wash the column with 400 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.
   (D2) In an nuclease-free tube, add 5 µl **DNase I** (1 U/µl)*, 35 µl **DNA Digestion Buffer** and mix. Add mixture directly into the column matrix.
   (D3) Incubate the column at room temperature (20-30°C) for 15 minutes.
9. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
11. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).
12. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge. The eluted RNA can be used immediately or stored frozen.

    Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

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1 Up to 1 ml whole blood can be processed per prep, with reloading. Adjust volumes proportionally (steps 1-3), if needed.
2 To process samples > 700 µl, columns may be reloaded.
3 For processing large volumes, the vacuum manifold can be used. After loading, centrifuge the column to remove residual liquid.
4 Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 4). * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/ml of reaction mixture at 25°C.

Appendices

RNA Purification (pelleted blood cells)

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ This protocol is compatible with pelleted blood cells (PBMCs, WBCs (e.g., after RBC lysis[1]), buffy coat, and PAXgene™ Blood RNA Tube cell pellet).

1. Resuspend the pelleted cells with 300 µl **DNA/RNA Shield™** (1X)[2].
2. Add 30 µl **PK Digestion Buffer** and 15 µl **Proteinase K** to the sample and mix well. Then incubate at 55°C for 30 minutes[3]
3. After incubation, vortex the sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer the aqueous supernatant into a nuclease-free tube (not provided).
4. Add an equal volume of **RNA Recovery Buffer** to the sample (1:1) and mix by well.

   Example: Add 300 µl **RNA Recovery Buffer** to 300 µl supernatant.
5. Transfer the mixture into a **Zymo-Spin™ IIICG Column**[4] (**green**) in a **Collection Tube** and centrifuge[3]. Save the flow-through!
6. To the flow-through (step 5), add an equal volume of ethanol (95-100%) (1:1) and mix well.

   Example: Add 600 µl ethanol to 600 µl flow-through.
7. Transfer the mixture into a **Zymo-Spin™ IC Column**[4] in a **Collection Tube** and centrifuge. Discard the flow-through.
8. Perform **DNase I** treatment (recommended; see page 5).
9. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
11. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).
12. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge. The eluted RNA can be used immediately or stored frozen.

    Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

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1 Red Blood Cell Lysis: Add 600 µl RBC Lysis Buffer (R1022-2-50) to each 200 µl whole blood and mix by inverting. Incubate for 5 minutes at room temperature. Then centrifuge for 1 minute to pellet cells. Discard the supernatant and proceed to step 1.
2 To prepare a 1X solution, add an equal volume of nuclease-free water (not provided) to **DNA/RNA Shield™** (2X concentrate) (1:1) and mix.
3 Optimal incubation times may vary.
4 To process samples > 700 µl, columns may be reloaded.

(Appendices continued)

RNA Purification (nucleated whole blood)

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ This protocol is for processing up to 50 µl nucleated whole blood (chicken, reptilian, etc.)

1. Add 1 ml of **DNA/RNA Shield** (1X)[1] to 50 µl nucleated whole blood sample and mix by pipetting up and down/vortexing. Centrifuge to reduce foam.
2. Add 800 µl **PK Digestion Buffer** and 20 µl **Proteinase K** for each 50 µl blood sample and mix well. Then incubate at 55°C for 30 minutes[2].
3. Continue with the RNA Purification (whole blood) protocol, page 5, step 3.

1 To prepare a 1X solution, add an equal volume of nuclease-free water (not provided) to **DNA/RNA Shield**™ (2X concentrate) (1:1) and mix.
2 Optimal incubation times may vary.

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ Whole Blood	R1201	50 preps.

Individual Kit Components	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 ml
DNase/RNase-Free Water	W1001-10 W1001-30	10 ml 30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
PK Digestion Buffer	R1200-1-5 R1200-1-20	5 ml 20 ml
Proteinase K (lyophilized) & Storage Buffer	D3001-2-5 D3001-2-20	5 mg 20 mg
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Zymo-Spin™ IC Columns	C1004-50	50
Collection Tubes	C1001-50	50
RNA Recovery Buffer	R1070-1-10	10 ml

Complete Your Workflow

✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

✓ For isolation of RNA from any sample:

Quick-RNA kits	
Microprep #R1050	From 1 cell and up
MagBeads #R2132/R2133	Automatable (Tecan, Hamilton, Kingfisher, etc.)

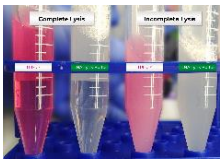
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input: - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image). 
Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	Sample handling: - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. Incomplete lysis and/or cellular debris: - Increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
Low yield	Sample input: - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised nucleic acid recovery. Use less input material and/or increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer. High-protein content (blood, plasma/serum, etc.) - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment or perform DNase I treatment post-purification, then re-purify the treated sample. - For future preps, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into DNA/RNA Shield™ and/or RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes

Notes

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This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. 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.

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