

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™ IICG Columns	50
Zymo-Spin™ IC Columns	50
Collection Tubes	100
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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.

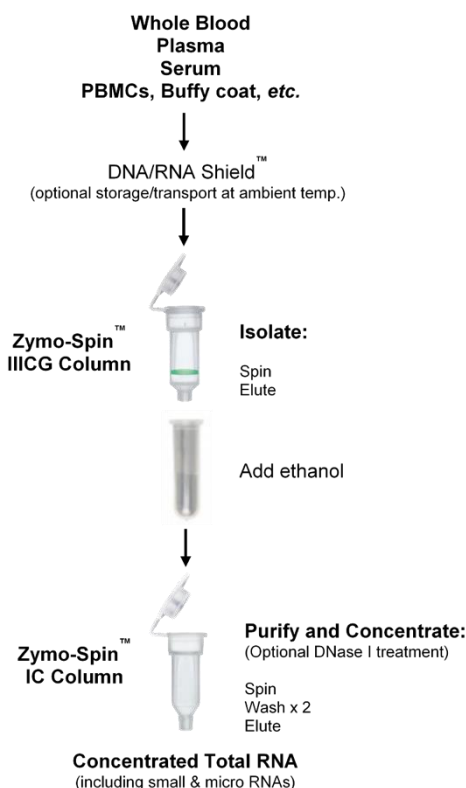
Specifications

- **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 $\geq 6 \mu\text{l}$ 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**
#E1011-A (1500 U), add 1,500 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 blood¹ (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 centrifuge³. 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.

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 A₂₆₀ 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 lysis¹), 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 minutes³
 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 Column⁴** (green) in a **Collection Tube** and centrifuge³. 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 Column⁴** 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.

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 minutes².
 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

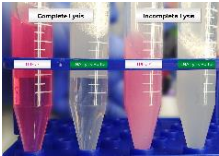
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

- ✓ 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

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Notes

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