ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ Whole Blood

Catalog Nos. **R1201**

Highlights

- Purify high-quality total RNA (including small/micro RNAs) from whole and partitioned blood samples.
- Compatible with commonly used anticoagulants (*i.e.,* EDTA, citrate, heparin).
- *DNA-free* RNA is ready for use in any downstream application. *DNase I included*.
- Worry-free sample storage at ambient temperatures with provided DNA/RNA Shield™.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

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.

For assistance, contact us at tech@zymoresearch.com

Product Contents

***Quick-RNA*™ Whole Blood** (Kit Size)	**R1201** (50 Preps.)
DNA/RNA Shield™ (2X concentrate)	2x 25 ml
RNA Recovery Buffer	10 ml
RNA Prep Buffer	25 ml
RNA Wash Buffer[1] (concentrate)	24 ml
DNase/RNase-Free Water	4 ml
DNase I[2] (lyophilized)	1
DNA Digestion Buffer	4 ml
PK Digestion Buffer	5 ml
Proteinase K[3] & Storage Buffer	2x 20 mg
Zymo-Spin™ IIICG Columns	50
Zymo-Spin™ IC Columns	50
Collection Tubes	2x 50
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Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.

[1] Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
[2] Prior to use, reconstitute the lyophilized **DNase** I with 275 µl **DNase/RNase-Free Water**. Mix by gentle inversion. Store aliquots at -20ºC.
[3] Prior to use, reconstitute each lyophilized **Proteinase K** with 1040 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20ºC.

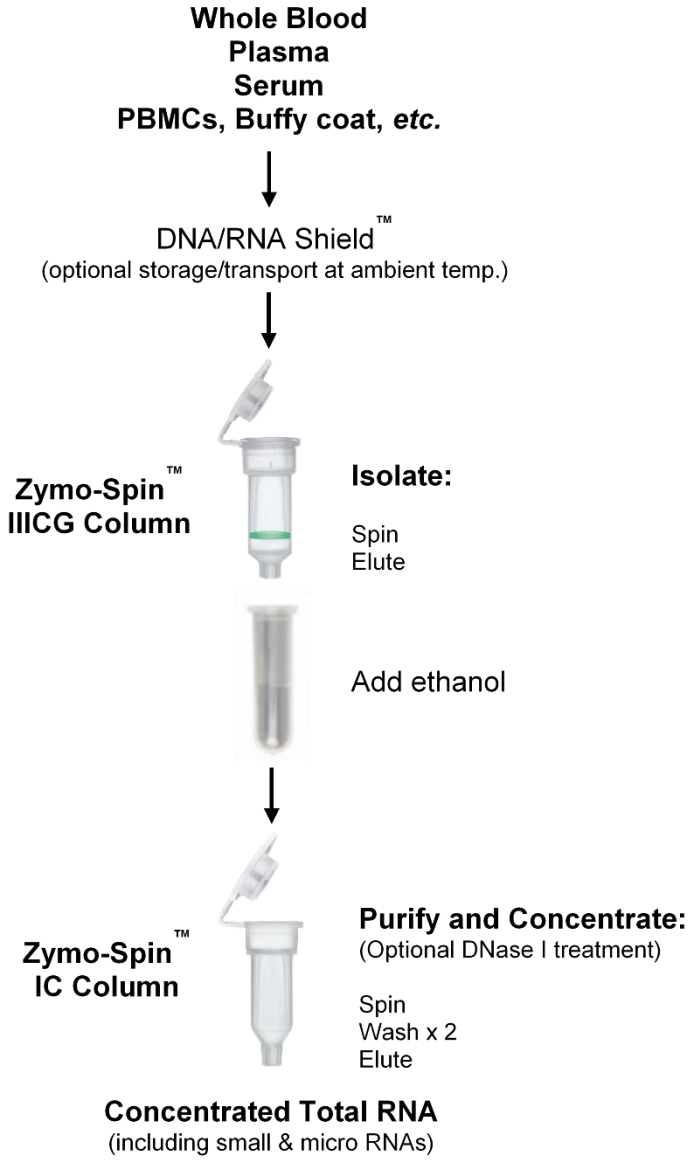
RBC Lysis Buffer (R1022-2-50, R1022-2-100) available separately for lysing red blood cells from fresh whole blood.

Specifications

- **Sample Sources** – Up to 1 ml mammalian whole blood (fresh or stored in **DNA/RNA Shield**™ 2X concentrate), plasma, or serum. Also compatible with pelleted blood cells (PBMCs, WBCs, buffy coat, pelleted samples from PAXgene™ Blood RNA Tube, *etc.*) and nucleated blood.
- **Sample Preservation** – **DNA/RNA Shield**™ effectively lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures.
- **RNA Size** – RNAs ≥17 nucleotides.
- **RNA Purity** – A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8. DNase I provided for complete removal of DNA.
- **RNA Recovery** –RNA yields are species and sample/donor dependent and averages can range from 2-10 µg (1 ml human blood).
- **RNA Storage** – RNA eluted with ≥6 µl RNase-free water can be stored at ≤-70 ºC.
- **Equipment Needed** – Microcentrifuge and vortex.

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** and then purified and concentrated on the **Zymo-Spin™ IC Column**. RNA is eluted into ≥6 µl of RNase-free water and is ready for any downstream application including RT-PCR, sequencing, *etc.*



For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Notes:

Ensure the RNA isolation procedure is performed in an RNase-free environment.

The lyophilized **Proteinase K** and **DNase I** are stable as shipped.

[1] This protocol is based on a 200 µl sample volume.

With reloading, up to 1 ml blood per prep can be processed.

[2] To process samples >700 µl, **Zymo-Spin**™ columns may be reloaded.

[3] For processing large volumes, a vacuum manifold can be used in place of centrifugation for this step. Following the vacuum step, centrifuge the column to remove residual liquid.

[4] 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.*

RNA Purification from pelleted WBCs, buffy coat, or PAXgene™ samples (page 4).

RNA Purification from nucleated blood (page 5).

Reagent Preparation

- ✓ Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
- ✓ Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** at 1 U/µl. Mix by gentle inversion. Store frozen aliquots at -20ºC.
- ✓ Add 1040 µl **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store at -20ºC.

RNA Purification[1]: Whole Blood (mammalian)

All centrifugation steps should be performed at 10,000-16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Add 200 µl **DNA/RNA Shield**™ (2X concentrate) to 200 µl whole blood and mix thoroughly.
2. Add 8 µl **Proteinase K** and mix. Incubate at room temperature (20-30ºC) for 30 minutes.
3. Add an equal volume of isopropanol and mix by vortexing.
4. Transfer the sample into a **Zymo-Spin**™ **IIICG Column**[2] in a **Collection Tube** and centrifuge[3]. Transfer the column into an RNase-free tube (not provided).
5. Add 200 µl **RNA Recovery Buffer** to the **Zymo-Spin**™ **IIICG Column** and centrifuge.
6. Add 200 µl ethanol (95-100%) to the flow-through (step 5) and mix well.
7. Transfer the mixture into a **Zymo-Spin**™ **IC Column**[2] in a **Collection Tube** and centrifuge. Discard the flow-through.

   Recommended: **DNase I** treatment (in-column)[4]:

   (D1) Wash the column with 400 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.
   (D2) In an RNase-free tube, add 5 µl **DNase I** (1 U/µl)*, 35 µl **DNA Digestion Buffer** and mix by inversion. Add the mix directly to the column matrix.
   (D3) Incubate the column at room temperature (20-30ºC) for 15 minutes. Proceed to step 8.

8. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
9. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into an RNase-free tube (not provided).
11. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

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

    Eluted RNA can be used immediately or stored at ≤-70ºC.

Ordering Information

Product Description	Catalog No.	Kit Size
***Quick*-RNA™ Whole Blood**	R1201	50 Preps.

For Individual Sale	Catalog No.	Amount
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
RBC Lysis Buffer	R1022-2-50 R1022-2-100	50 ml 100 ml
RNA Recovery Buffer	R1070-1-10	10 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
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
PK Digestion Buffer	R1200-1-5 R1200-1-20	5 ml 20 ml
Proteinase K (lyophilized) (supplied with Proteinase K Storage Buffer)	D3001-2-5 D3001-2-20	5 mg set 20 mg set
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

Notes:

[1] **Red Blood Cell Lysis:** Add 600 µl **RBC Lysis Buffer** (R1022-2-50) to each 200 µl fresh whole blood in an RNase-free tube and mix by inverting. Incubate 5 minutes at room temperature and centrifuge at ≥12,000 *x g* for 1 minute to pellet cells. Remove the supernatant and proceed to Step 1.

[2] To prepare 1X solution, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided).

[3] Optimal incubation times may vary.

[4] To process samples >700 µl, **Zymo-Spin™** columns may be reloaded.

[5] 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.*

Appendices

RNA Purification from Pelleted Blood Cells

All centrifugation steps should be performed at 10,000-16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

This protocol is compatible with pelleted blood cells including PBMCs, WBCs (*e.g.* after RBC lysis[1]), buffy coat, pelleted samples from PAXgene™ Blood RNA Tube, *etc*.

1. Resuspend the pelleted samples using 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. Incubate at 55ºC for 30 minutes[3].
3. After incubation, vortex sample and then centrifuge at max speed for 2 minutes to pellet debris. Transfer the aqueous supernatant into an RNase-free tube (not provided).
4. Add 1 volume **RNA Recovery Buffer** to the sample and mix well.
5. Transfer the sample into a **Zymo-Spin™ IIICG Column**[4] in a **Collection Tube** and centrifuge.

   Save the flow-through.
6. Add 1 volume ethanol (95-100%) to the flow-through and mix well.
7. Transfer the mixture into a **Zymo-Spin™ IC Column**[4] in a **Collection Tube** and centrifuge. Discard the flow-through.

   Recommended: **DNase I** treatment (in-column)[5]:

   (D1) Wash the column with 400 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.
   (D2) In an RNase-free tube, add 5 µl **DNase I** (1 U/µl)*, 35 µl **DNA Digestion Buffer** and mix by inversion. Add the mix directly to the column matrix.
   (D3) Incubate the column at room temperature (20-30ºC) for 15 minutes. Proceed to step 8.
8. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
9. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into an RNase-free tube (not provided).
11. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

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

    Eluted RNA can be used immediately or stored at ≤-70ºC.

RNA Purification from Nucleated Whole Blood

All centrifugation steps should be performed at 10,000-16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) 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 of nucleated blood sample and mix by pipetting up and down and 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. Incubate at 55ºC for 30 minutes[2].
3. Continue with Page 3, Step 3 and proceed with the protocol to purify RNA.

Notes:

[1] To prepare 1X solution, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided).

[2] Optimal incubation times may vary.

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