ZYMO RESEARCH

RNA Purification Made Simple™

Quick-DNA/RNA™ Blood Tube Kit

DNA & RNA from DNA/RNA Shield™ Blood Collection Tube

Highlights

- Spin-column purification of DNA and/or total RNA (including small/microRNAs) from blood samples collected and stored in the **DNA/RNA Shield™ Blood Collection Tube** (R1150).
- Direct sample processing - no reagent removal and no pelleting necessary.
- High-quality DNA and/or RNA is ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:
R1151

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

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Product Contents

Quick-DNA/RNA™ Blood Tube Kit	R1151 (50 prep)
DNA/RNA Prep Buffer	50 ml
DNA/RNA Wash Buffer[1] (concentrate)	24 ml
RNA Recovery Buffer	10 ml
DNA Recovery Buffer[2] (concentrate)	9 ml
DNase/RNase-Free Water	6 ml
DNase I[3] (lyophilized)	250 U
DNA Digestion Buffer	4 ml
Proteinase K[4] (lyophilized) & Storage Buffer	125 mg
Reservoir (25 ml)	50
Zymo-Spin™ IIICG Columns	50 (x2)
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 **DNA/RNA Wash Buffer** concentrate.
2 Add 6 ml ethanol (95-100%) to the 9 ml **DNA Recovery Buffer** concentrate.
3 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**
4 Add **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 125 mg, see Buffer Preparation, page 4. Store frozen aliquots.

Specifications

- **Sample Sources –** For use with the **DNA/RNA Shield™ - Blood Collection Tube** (prefilled with 6 ml DNA/RNA Shield™) for the direct collection of up to 3 ml whole blood (human or animal).

- **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 (page 8).

- **Size –** Capable of recovering DNA and total RNA including small/microRNAs (≥ 17 nt).

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

- **Binding Capacity – Zymo-Spin™ IIICG Column** yield up to 50 µg DNA and up to 100 µg RNA.

- **Average DNA/RNA Yield –** Sample yield is species and donor dependent. Average DNA yield is 15-30 µg and average RNA yield is 6-30 µg from 3 ml human blood.

- **Elution Volume –** ≥ 50 µl **DNase/RNase-Free Water.**

- **Equipment** (user provided) **–** Microcentrifuge, vortex, vacuum manifold (optional).

  This protocol is compatible with any conventional vacuum-based manifold. The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure.

- **Materials** (available separately) **–** DNA/RNA Shield™ – Blood Collection Tube (R1150; 50 pack).

Product Description

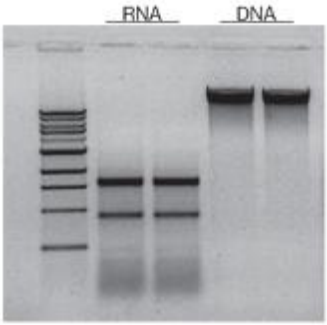
The **Quick-DNA/RNA™ Blood Tube Kit** is designed for use with the **DNA/RNA Shield™ - Blood Collection Tube** (R1150; available separately), enabling worry-free sample storage at ambient temperatures.

The purification procedure uses Zymo-Spin™ column technology. Simply bind the sample onto the **Zymo-Spin™ IIICG Column** with the aid of reservoirs (vacuum compatible). There is no reagent removal and no pelleting for easy direct whole tube processing.

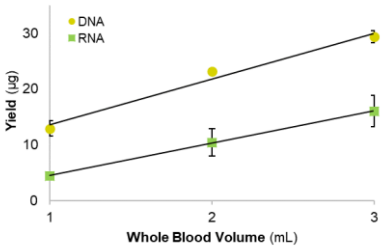
High-quality DNA and/or total RNA from 3 ml whole blood is eluted into ≥ 50 µl of **DNase/RNase-Free Water** and is ready for any downstream application including RT/qPCR, sequencing, etc.

Isolate DNA and/or RNA from blood samples collected in the **DNA/RNA Shield™ - Blood Collection Tube (R1150)**

High-Quality Nucleic Acid without Reagent Removal



High-quality DNA and RNA is effectively purified from blood stored in DNA/RNA Shield™. High molecular weight DNA was intact with no apparent degradation. Also, RNA was high quality, DNA-free and included small RNAs.



Linear recovery of DNA and RNA using the Quick-DNA/RNA™ Blood Tube Kit. Aliquots (1-3 ml) of whole blood stored in DNA/RNA Shield™ were used for purification (n=3).

Protocol

The protocol consists of:
(I) Buffer Preparation, (II) DNA Purification, (III) RNA Purification and (IV) DNA & RNA Purification

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
- ✓ Add 6 ml ethanol (95-100%) to the 9 ml **DNA Recovery 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 6.5 ml **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 125 mg Vortex to dissolve and store frozen aliquots.

(II) DNA Purification

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

1. Transfer the contents of the **DNA/RNA Shield™ - Blood Collection Tube** to a 50 ml tube (not provided). If frozen, thaw the tube at room temperature and mix well.
2. Add 120 µl **Proteinase K** to the tube and mix by vortexing. Incubate at room temperature (20-30°C) for 30 minutes.
3. Add 9 ml isopropanol and mix by vortexing.
4. Assemble the **Reservoir (25 ml)** with the **Zymo-Spin™ IIICG Column** and place onto a vacuum manifold[1]. Add the mixture into the reservoir and turn on the vacuum until all of the liquid has passed completely through the column.
5. Remove the reservoir and place the column into a **Collection Tube**. Centrifuge to remove residual liquid.
6. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
7. Add 200 µl **RNA Recovery Buffer** to the column, let stand for 5 minutes and centrifuge. Discard the flow-through.
8. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
9. Add 400 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 200 µl **DNA Recovery Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the buffer. Carefully transfer the column into a nuclease-free tube (not provided).
11. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix, let stand for 5 minutes, and then centrifuge.

    Alternatively, for highly concentrated DNA use ≥ 50 µl elution.
    The eluted DNA can be used immediately or stored frozen.

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1 Alternatively, to process samples > 700 µl by microcentrifuge, columns may be reloaded. Proceed to step 6.

(III) RNA Purification

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

1. Transfer the contents of the **DNA/RNA Shield™ - Blood Collection Tube** to a 50 ml tube (not provided). If frozen, thaw the tube at room temperature and mix well.

2. Add 120 µl **Proteinase K** to the tube and mix by vortexing. Incubate at room temperature (20-30°C) for 30 minutes.

3. Add 9 ml isopropanol and mix by vortexing.

4. Assemble the **Reservoir (25 ml)** with the **Zymo-Spin™ IIICG Column** and place onto a vacuum manifold[1]. Add the mixture into the reservoir and turn on the vacuum until all the liquid has passed completely through the column.

5. Remove the reservoir and place the column into a **Collection Tube**. Centrifuge to remove residual liquid.

6. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

7. **DNase I**[2] 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)*, 75 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.

8. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

9. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

10. Add 400 µl **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into a new microcentrifuge tube (not provided).

11. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

    Alternatively, for highly concentrated RNA use ≥ 50 µl elution.
    The eluted RNA can be used immediately or stored frozen.

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1 Alternatively, to process samples > 700 µl by microcentrifuge, columns may be reloaded. Proceed to step 6.
2 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.

(IV) DNA & RNA Purification (in separate fractions)

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

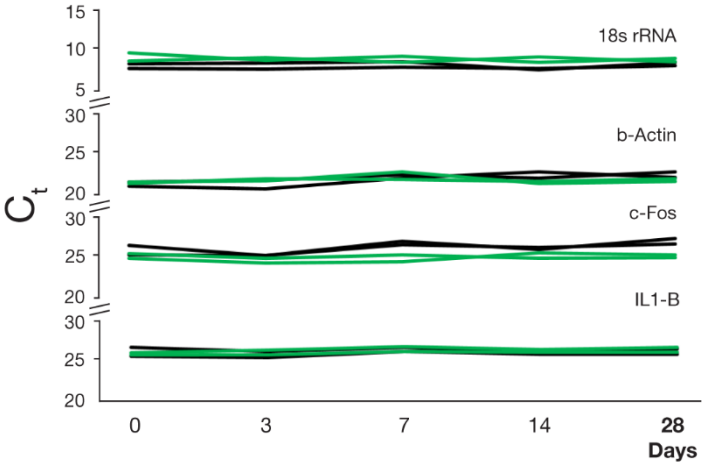
1. Transfer the contents of the **DNA/RNA Shield™ - Blood Collection Tube** to a 50 ml tube (not provided). If frozen, thaw the tube at room temperature and mix well.
2. Add 120 µl **Proteinase K** to the tube and mix by vortexing. Incubate at room temperature (20-30°C) for 30 minutes.
3. Add 9 ml isopropanol and mix by vortexing.
4. Assemble the **Reservoir (25 ml)** with the **Zymo-Spin™ IIICG Column** and place onto a vacuum manifold[1]. Add the mixture into the reservoir and turn on the vacuum until all of the liquid has passed completely through the column.
5. Remove the reservoir and place the column into a **Collection Tube**. Centrifuge to remove residual liquid.
6. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through. Then transfer the column into a new nuclease-free tube (not provided).
7. Add 200 µl **RNA Recovery Buffer** directly to the column matrix, let stand for 5 minutes and then centrifuge. Save the flow-through!

8. **DNA Purification** **(DNA is bound to the column)**	8. **RNA Purification** **(RNA is in the flow-through)**
a. Transfer the column into a new **Collection Tube**.	a. To the flow-through, add an equal volume of ethanol (95-100%) (1:1) and mix well. Example: Add 200 µl ethanol to 200 µl flow-through.
b. Continue with the page 5, step 8.	b. Transfer the mixture into a new **Zymo-Spin™ IIICG Column** in a **Collection Tube** and centrifuge. Discard the flow-through. At this point, DNase I treatment can be performed. See page 6, step 7.
	c. Continue with page 6, step 8.

1 Alternatively, to process samples > 700 µl by microcentrifuge, columns may be reloaded. Then proceed to step 6.

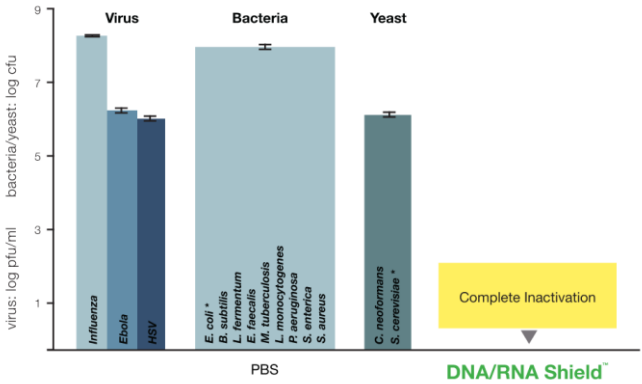
Appendices

Nucleic Acid Stabilization at Ambient Temperature in Human Blood



RNA in blood is effectively stabilized in DNA/RNA Shield™ at ambient temperature. Graph shows cellular RNA from human whole blood stabilized in **DNA/RNA Shield™** at the indicated time points and analyzed by (RT)qPCR.

Microbial Inactivation



Viruses, bacteria and yeast are effectively inactivated by DNA/RNA Shield™. Samples containing the infectious agent (virus, bacteria, yeast) were treated with **DNA/RNA Shield™** or mock (PBS) treated for 5 minutes. CFU was separately quantified and titer (PFU) was subsequently determined by plaque assay.

Ordering Information

Product Description	Catalog No.	Size
Quick-DNA/RNA™ Blood Tube Kit	R1151	50 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Shield™ – Blood Collection Tube	R1150	50
DNA/RNA Prep Buffer	D7010-2-25 D7010-2-50	25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-12 D7012-3-24	12 ml 24 ml
DNase/RNase-Free Water	W1001-6 W1001-10	6 ml 10 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Proteinase K (lyophilized) & Storage Buffer Set	D3001-2-20 D3001-2-125	20 mg 125 mg
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Collection Tubes	C1001-50	50
Reservoir (25 ml)	C1039-25	25

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-DNA/RNA kits	
Microprep Plus #D7005	From 1 cell and up
MagBeads #R2130/R2131	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
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.
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. High-protein content (blood, plasma/serum, etc.) - Perform Proteinase K treatment to the sample prior to purification.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment (page 6) or perform DNase I treatment post-purification, then re-purify the treated sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into DNA/RNA Shield™. 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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Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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.

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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