INSTRUCTION MANUAL

Quick-DNA/RNA™ Blood Tube Kit

Catalog No. **R1151**

Highlights

- For use with DNA/RNA Shield™ - Blood Collection Tube (R1150)
- No reagent removal (no pelleting) – Direct sample processing.
- Purify high quality DNA and/or total RNA (including small/micro RNAs) from the same whole blood sample. Ready for use in any downstream application. *DNase I included*.

Contents

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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*-DNA/RNA™ Blood Tube Kit** (Kit Size)	**R1151** (50 Preps.)
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)	1
DNA Digestion Buffer	4 ml
Proteinase K[4] & Storage Buffer	125 mg
Zymo-Spin™ IIICG Columns	50
Reservoir (25 mL)	50
Collection Tubes	100
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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 **DNA/RNA Wash Buffer** concentrate.
[2] Before starting, add 6 ml ethanol (95-100%) to the 9 ml **DNA Recovery Buffer** concentrate.
[3] Prior to use, reconstitute the lyophilized **DNase** I with 275 µl **DNase/RNase-Free Water**. Mix by gentle inversion. Store aliquots at -20ºC.
[4] Prior to use, reconstitute the lyophilized **Proteinase K** with 6.5 ml **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20ºC.

Specifications

For processing a smaller aliquot of sample (*e.g.* 0.5 ml blood), see the *Quick*-RNA™ Whole Blood (R1201).

- **Sample Sources** – For use with **DNA/RNA Shield™ - Blood Collection Tube** (prefilled with 6 mL of DNA/RNA Shield™) for the direct collection of up to 3 ml whole-blood (human or animal).
- **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.
- **Size Limits** – Capable of recovering DNA and total RNA ≥17 nucleotides.
- **Purity** – High quality DNA and RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) are recovered.
- **Recovery** – Yields are species and sample/donor dependent. DNA yields average from 15-30 µg and RNA yields average from 6-30 µg (3 ml human blood).
- **Storage** – DNA and RNA eluted with **DNase/RNase-Free Water** can be stored at ≤-70 ºC. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, and vacuum/vacuum manifold (recommended).

Product Description

The ***Quick*-DNA/RNA™ Blood Tube Kit** is designed for use with the **DNA/RNA Shield™ - Blood Collection Tube** (R1150), 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-PCR, sequencing, *etc.*

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

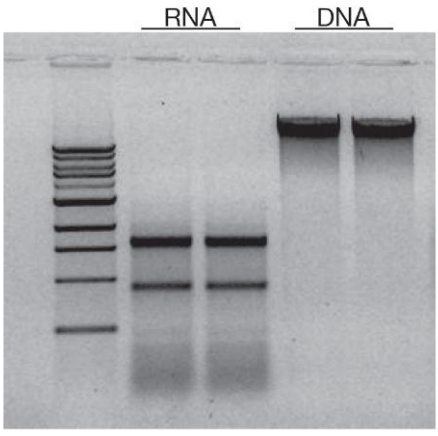
Purification Guide

DNA
Page 4

RNA
incl. small & micro RNAs
Page 5

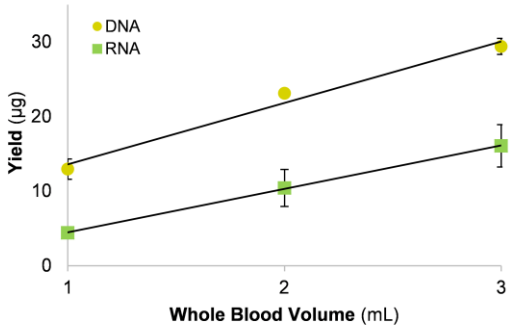
DNA & RNA
from the same sample
Page 6

High Quality Nucleic Acid without Reagent Removal



High quality DNA and RNA is effectively purified from blood stored in DNA/RNA Shield™.

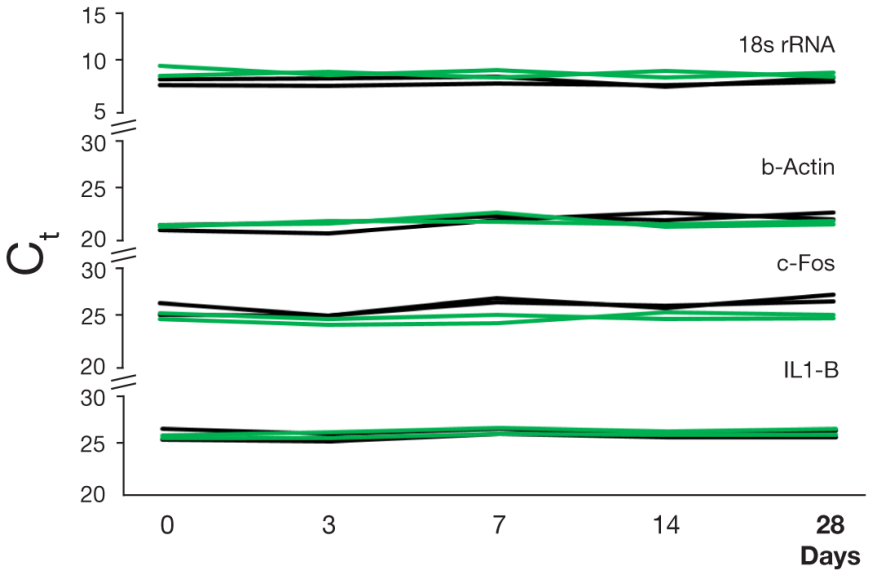
High molecular weight DNA was intact with no apparent degradation. Also, RNA was high quality, DNA-free and included small RNAs.



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).

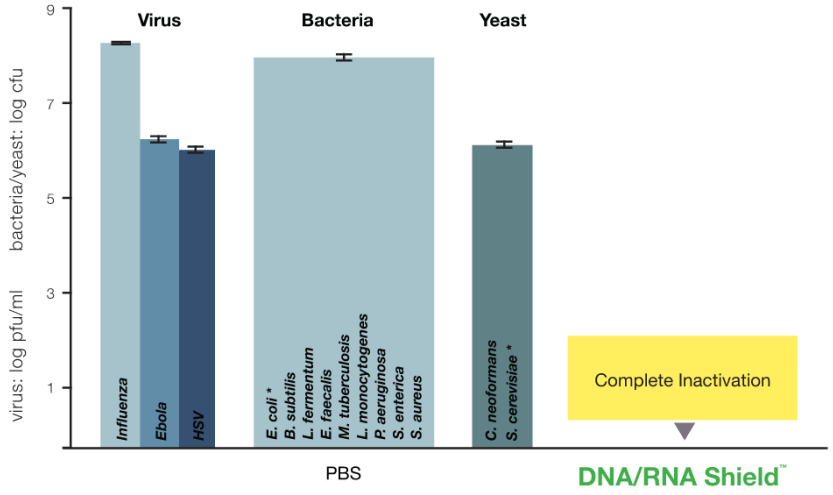
Nucleic Acid Stabilization at Ambient Temperature In Human Blood



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.

Reagent Preparation

- ✓ Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
- ✓ Before starting, add 6 ml ethanol (95-100%) to the 9 ml **DNA Recovery 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 6.5 ml **Proteinase K Storage Buffer** to reconstitute the lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store at -20ºC.

Notes:

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

Protocols

The protocols consists of DNA Purification (page 4), RNA Purification (page 5), or DNA & RNA Purification (page 6).

This product 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 at the vacuum manifold.

DNA Purification

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. 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.
2. Add 120 µl **Proteinase K** to the tube and mix by vortexing. Incubate at room temperature (20-30ºC) 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 new microcentrifuge 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.

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

[1] Alternative protocol: a microcentrifuge can be used. Transfer 700 µl sample to the column and centrifuge. Discard the flow-through. Reload until all liquid is passed through. Transfer the column into a new Collection Tube. Proceed to Step 6.

Notes:

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

[1] Alternative protocol: a microcentrifuge can be used. Transfer 700 µl sample to the column and centrifuge. Discard the flow-through. Reload until all liquid is passed through. Transfer the column into a new Collection Tube. Proceed to Step 6.

[2] 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

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

   Recommended: **DNase I** treatment (in-column)[2]:

   (D1) Wash the column with 400 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.
   (D2) In an RNase-free tube, add 5 µl **DNase I** (1 U/µl)*, 75 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 7.
7. Add 400 µl **DNA/RNA Prep Buffer** to the column 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** 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).
10. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

    Alternatively, for highly concentrated RNA use ≥50 µl elution.

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

DNA & RNA Purification

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. 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.
2. Add 120 µl **Proteinase K** to the tube and mix by vortexing. Incubate at room temperature (20-30ºC) 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 though 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. Transfer into a clean microcentrifuge tube (not provided). Add 200 µl **RNA Recovery Buffer** directly to the column matrix, let stand 5 minutes and then centrifuge.

   Save the flow-through.

DNA Purification

(DNA is bound to the column)

8. Transfer the **Zymo-Spin™ IIICG Column** into a new **Collection Tube**.
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** to the column and centrifuge. Discard the flow-through.
11. 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 clean microcentrifuge tube.

RNA Purification

(RNA is in the flow-through)

8. Add 1 volume ethanol (95-100%) to the flow-through and mix well. Then transfer the sample into a new **Zymo-Spin™ IIICG Column** in a **Collection Tube** and centrifuge. Discard the flow-through.[2]
9. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
11. 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 clean microcentrifuge tube.

12. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix, let stand for 5 minutes, and then centrifuge to elute DNA and RNA from the respective column.

    Alternatively, for highly concentrated DNA and RNA use ≥50 µl elution.

    The eluted DNA & RNA can be used immediately or stored at ≤-70ºC.

Notes:

[1] Alternative protocol: a microcentrifuge can be used. Transfer 700 µl sample to the column and centrifuge. Discard the flow-through. Reload until all liquid is passed through. Transfer the column into a new Collection Tube. Proceed to Step 6.

[2] At this point, RNA samples can be in-column DNase I treated (page 5).

Ordering Information

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

For Individual Sale	Catalog No.	Amount
DNA/RNA Shield™ - Blood Collection Tube	R1150	50 Pack
DNA/RNA Prep Buffer	D7010-2-10 D7010-2-25 D7010-2-50	10 ml 25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-6 D7010-3-12 D7010-3-24	6 ml 12 ml 24 ml
RNA Recovery Buffer	R1070-1-10	10 ml
DNA Recovery Buffer (concentrate)	R2050-5-9	9 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
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
Reservoir (25 mL)	C1039-25	25
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

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The Beauty of Science is to Make Things Simple