omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® PX Blood RNA Kit

R1057-00	5 preps
R1057-01	50 preps

Manual Date: November 2018
Revision Number: v6.0

For Research Use Only

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Introduction and Overview

E.Z.N.A.® PX Blood RNA Kit is designed for isolation of total RNA from blood samples stored in preservation reagents and PAXgene® Blood RNA Tubes. This procedure completely removes contaminants and enzyme inhibitors producing high-quality RNA. RNA purified using the E.Z.N.A.® PX Blood RNA Kit is ready for applications such as RT-PCR.

The samples are removed from the preservation reagents. For blood samples stored in PAXgene® Blood RNA Tubes, the cells are collected by centrifugation. Samples are washed and lysed under an optimized buffer containing Proteinase K. The samples are transferred to a Homogenizer Mini Column to remove cell debris and other particulates. After adjusting the binding conditions with ethanol, the samples are loaded on the HiBind® RNA Mini Column. With a brief centrifugation or vacuum step, the samples pass through the column matrix which binds the RNA. Genomic DNA is removed with an on-the-column DNase I digestion treatment. After three wash steps, purified RNA is eluted with RNase-free water.

New in this Edition:

November 2018:
- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) has been discontinued and is no longer available to purchase.

February 2018:
- DNase Digestion Buffer storage temperature changed from room temperature to -20°C.

June 2015:
- NTL Lysis Buffer has replaced TRK Lysis Buffer to increase yield and RIN value.

Kit Contents

Product	R1057-00	R1057-01
Purifications	5	50
HiBind® RNA Mini Columns	5	50
Homogenizer Mini Columns	5	50
2 mL Collection Tubes	15	150
NTL Lysis Buffer	2 mL	25 mL
RWF Buffer	5 mL	50 mL
RNA Wash Buffer II	2.5 mL	12 mL
Proteinase K Solution	250 µL	2.2 mL
DNase I Digestion Buffer	1 mL	5 mL
DNase I	9 µL	78 µL
Nuclease-free Water	60 mL	250 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® PX Blood RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. DNase I and DNase I Digestion Buffer should be stored at -20°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in NTL Lysis Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R1057-00	10 mL
R1057-01	48 mL

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.
- Prepare all materials required before starting the procedure to minimize RNA degradation.
- Optional: 2-mercaptoethanol is key in denaturing RNases and can be added to an aliquot of NTL Lysis Buffer before use. Add 20 µL 2-mercaptoethanol per 1 mL NTL Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. Nuclease-free Water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

E.Z.N.A.® PX Blood RNA Kit Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x *g*
- 100% ethanol
- RNase-free filter pipette tips
- RNase-free water
- 1.5 or 2.0 mL microcentrifuge tubes
- Shaking incubators or heat blocks capable of 55°C, 65°C, and 70°C
- PAXgene® Blood RNA Tubes
- Centrifuge with swing-bucket rotor capable of 5,500 x *g*
- Optional: 2-mercaptoethanol

Before Starting:

- Prepare RNA Wash Buffer II as instructed in the "Preparing Reagents" section located on Page 4.
- Heat the incubators or heat blocks to 55°C, 65°C, and 70°C.
- Optional: Prepare NTL Lysis Buffer with 2-mercaptoethanol as instructed on Page 5.

1. Centrifuge the PAXgene® Blood RNA Tube for 10 minutes at 3,000-5,000 x *g*.

2. Aspirate and discard the supernatant.

3. Add 4 mL RNase-free water. Vortex to completely resuspended the pellet.

4. Centrifuge the PAXgene® Blood RNA Tube for 10 minutes at 3,000-5,000 x *g*.

5. Aspirate and discard the supernatant.

   Note: Incomplete removal of the supernatant will reduce the lysis efficiency and dilute the lysate, thereby reducing the RNA yield.

6. Add 485 µL Nuclease-free Water. Vortex to completely resuspended the pellet.

7. Transfer the sample into a new 1.5 mL microcentrifuge tube.

8. Add 375 µL NTL Lysis Buffer and 40 µL Proteinase K Solution. Vortex for 5 seconds to mix thoroughly.

   Optional: Add 20 µL β-mercaptoethanol per 1 mL NTL Lysis Buffer to aid in the denaturation of RNases.

9. Incubate at 55°C for 15 minutes using a shaking incubator.

10. Insert a Homogenizer Mini Column into a 2 mL Collection Tube.

11. Transfer the sample directly into the Homogenizer Mini Column.

12. Centrifuge at maximum speed (>13,000 x *g*) for 3 minutes.

13. Carefully transfer the entire filtrate supernatant to a new 1.5 mL microcentrifuge tube. Do not disturb the pellet in the 2 mL Collection Tube.

14. Add 0.5 volumes 100% ethanol. Vortex to mix thoroughly.

15. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.

16. Transfer 750 µL sample into the HiBind® RNA Mini Column.

17. Centrifuge at maximum speed for 1 minute.

18. Aspirate and discard the filtrate and reuse the collection tube.

19. Repeat Steps 16-18 until the remaining sample has been transferred to the HiBind® RNA Mini Column.

20. Insert a HiBind® RNA Mini Column into a new 2 mL Collection Tube.

21. Add 350 µL RWF Buffer.

22. Centrifuge at maximum speed for 1 minute.

23. Aspirate and discard the filtrate and the collection tube.

24. For each of the HiBind® RNA Mini Column, prepare the DNase I digestion reaction mix as follows:

Buffer	Volume per Prep
DNase I Digestion Buffer	73.5 µL
RNase-free DNase I (20 Kunitz units/µL)	1.5 µL
Total volume	75 µL

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. **Do not vortex the DNase I mixture.** Mix gently by inverting the tube.
- Freshly prepare DNase I stock solution right before RNA isolation.
- Standard DNase buffers are not compatible with on-membrane DNase I digestion. The use of other buffers may affect the binding of RNA to the HiBind® matrix and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

25. Pipet 75 µL DNase I digestion reaction mix directly onto the surface of the HiBind® RNA Mini Column.

    Note: Make sure to pipet the DNase I digestion mixture directly onto the membrane. DNase I digestion will not be complete if some of the mixture is retained on the wall or o-ring of the HiBind® RNA Mini Column.

26. Let sit at room temperature for 15 minutes.

27. Add 500 µL RWF Buffer.

28. Centrifuge at maximum speed for 1 minute.

29. Aspirate and discard the filtrate and reuse the collection tube.

30. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

31. Centrifuge at maximum speed for 1 minute.

32. Aspirate and discard the filtrate and reuse the collection tube.

33. Repeat Steps 30-32 for a second RNA Wash Buffer II wash step.

34. Centrifuge the empty HiBind® RNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

35. Transfer the HiBind® RNA Mini Column into a 1.5 mL microcentrifuge tube.

36. Add 50-70 µL Nuclease-free Water or RNase-free water directly onto the center of the membrane.

37. Let sit at room temperature for 1 minute.

38. Centrifuge at maximum speed for 2 minutes.

39. Store RNA at -70°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Little or no RNA eluted	Less blood volume in the starting sample	• Make sure that 2.5 mL whole blood is in the PAXgene Blood RNA Tube. • Repeat elution. • Preheat Nuclease-free Water to 70°C prior to elution. • Incubate for 5 minutes with Nuclease-free Water prior to elution. • Make sure to measure the concentration of RNA by using 10mM Tris-HCl, pH 7.5. Do not use water. • Reduce the quantity of starting material.
	RNA remains on the plate	
	RNA Mini Column is overloaded	
Problem	Cause	Solution
Clogged column	Incomplete lysis	• Mix thoroughly after addition of NTL Lysis Buffer. • Reduce the amount of starting material.
Degraded RNA	RNase contamination	• Check the buffer and make sure there is no RNase contamination. • Follow protocol closely, and work quickly. • Ensure not to introduce RNase during the procedure.
Problem in downstream applications	Salt carry-over during elution	• Ensure that RNA Wash Buffer II has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • RNA Wash Buffer II must be stored at room temperature. • Repeat RNA Wash Buffer II wash step.
	Inhibitors of PCR	• Use less starting material. • Prolong incubation with NTL Lysis Buffer to completely lyse cells.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
DNase/RNase-free microcentrifuge tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free microcentrifuge tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00
Nuclease-free Water, 1000 mL	PD092
RNase-free DNase Set, 1500 units	E1091
RNase-free DNase Set, 6000 units	E1091-02
RNA Wash Buffer II, 20 mL	PDR046

Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

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Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

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