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Introduction

E.Z.N.A.™ PX RNA Kit is designed for isolation of total RNA from blood samples stored in special preserve reagents and Paxgene™ tubes. The procedure completely removes contaminants and enzyme inhibitors making RNA isolation fast, convenient, and reliable.

RNA purified using the E.Z.N.A.™ PX RNA Kit method is ready for applications such as RT-PCR*.

Principle

The samples are first removed from the preserve reagents . For blood samples stored in Paxgene™ tubes, the cell are collected by centrifugation. Sample are then washed and lysed under optimized buffer contains Proteinase K. The samples are transfer to a Homogenizer ColumnPlate to remove the cell debris and other particles. After adjusting the binding conditions with ethanol, the samples are loaded to the HiBind RNA Column. With a brief centrifugation or vacuum, the samples pass through the plate and the RNA binds to the Hibind® matrix. Genomic DNA is then removed with an on column Dnase Digestion. After two wash steps, purified RNA can be eluted with RNase-free water.

Storage and Stability

All components in the E.Z.N.A.™ PX RNA Kit Kit should be stored at room temperature . During shipping and storage, crystals may form in the TRK Lysis Buffer, simply warm to 37°C to dissolve. All kit components are guaranteed for at least 12 months from date of purchase. DNase I enzyme should be stored at -20°C

*The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.
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Kit Contents

Product Number	R1057-00	R1057-01	R1057-02
Purification times	5	50	200
HiBind™ RNA Columns	5	50	200
Omega Homogenizer Columns	5	50	200
2 ml Collection Tubes	15	150	600
TRK Lysis Buffer	2 ml	25 ml	100 ml
RWC Wash Buffer	5 ml	50 ml	200 ml
RNA Wash Buffer II	2.5 ml	12 ml	50 ml
Proteinase K	6 mg	60 mg	4 x 60 mg
Proteinase Storage Buffer	260 **µl**	3 ml	12 ml
DNase I Digestion Buffer	500 **µl**	5 ml	20 ml
DNase I	9 **µl**	78 **µl**	4 x 78 **µl**
DEPC water	40 ml	250 ml	1050 ml
Instruction Manual	1	1	1

Important Notes

Please take a few minutes to read this booklet thoroughly to become familiar with the protocol. Prepare all materials required before starting to minimize RNA degradation. All centrifugation steps must be carride out at 22°C-25°C.

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.
- Under cool ambient conditions, crystals may form in TRK Lysis Buffer. This is normal and the bottle should be warmed to redissolve the salt.
- **Optional**: ß-mercaptoethanol (ß-mercaptoethanol) Add 20 **µl** of ß-Me per 1ml of TRK Lysis Buffer. TRK Buffer containing ß-Me can be stored at room temperature for 1 month.

Before Starting

IMPORTANT	RNA Wash Buffer II must be diluted with absolute ethanol (96-100%) before use and store at room temperature.	
	R1057-00	Add 10 ml absolute ethanol
	R1057-01	Add 48 ml absolute ethanol per bottle
	R1057-02	Add 200 ml absolute ethanol per bottle

Protocol for Isolation of RNA from whole Blood Collected in Paxgene Blood Tubes

Additional materials supplied by user:

- Centrifuge with rotor capable 13,000 x g
- 96-100% ethanol
- Optional: ß-Mercaptoethanol
- RNase-free filter pipette tips Disposable latex gloves
- RNase-Free water
- 1.5 or 2.0 mL Centrifuge tubes
- incubators or heat blocks preset at 55°C,65°C, and 70°C
- PaxGene™ Blood Tubes
- Centrifuge with Swing-bucket rotor capable 5500 x g

1. Centrifuge the PAXgene™ Blood RNA Tube for 10 minutes at 3000-5000 x g using a swing-bucket rotor.
2. Remove the supernatant by decanting or pipetting. Add 4 ml RNase-free water to the pellet, close the tube with a cap.
3. Vortex until the pellet is completely resuspended. Centrifuge at 3000-5000 x g for 10 minutes using a swing-bucket rotor. Remove and discard the entire supernatant. **Note: Incompletely removal of supernatant will reduce the lysis efficiency and dilute the lysate, and therefore reduce the RNA yield**
4. Add 485 **µl** of DEPC-water, vortex the sample until the pellet is completely dissolved.

5. Transfer the sample into a new 1.5 ml micro centrifuge tube, add 375 µl TRK Lysis Buffer and 40 µl Proteinase K (25mg/ml). Mix throughly by vortexing for 5 seconds. Incubate at 55°C for 15 minutes using a shaker-incubator.

6. Transfer the sample directly into a Homogenizer column placed into a 2 ml collection tube, centrifuge at maximum speed (>13,000 x g) for 3 minutes.

7. Carefully transfer the entire supernatant of the flow-through fraction to a new 1.5ml tube without disturbing the pellet in the 2ml collection tube.

8. Add 0.5 volume of absolute ethanol (96-100%) into each sample. Mix the sample throughly by vortexing.

9. Pipet 750µl of sample into the HiBind RNA column placed in a 2 ml collection tube. Centrifuge at 8,000-20,000 x g for 1 minute. Discard the flow-through and reuse the collection tube.

10. Place the HiBind RNA column into a 2 ml collection tube from the previous step.Transfer the remaining sample into the HiBind RNA column with pipettor.Centrifuge at 8,000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

11. Place the HiBind RNA column into 2 ml collection tube and add 350 µl RWC Wash buffer into the HiBind RNA column. Spin at 8,000-20,000 x g for 1 minute.Discard the flowthrough and collection tube.

12. For each of the HiBind RNA column , prepare the DNase I digestion reaction mix as follows:

OBI DNase I Digestion Buffer	73.5 µl
RNase-free DNase I (20 Kunitz unites/µl)	1.5 µl
Total volume	75 µl

Note:

a. DNase I is very sensitive for physical denaturaion, so do not vortex this DNase I mixture. Mix gently by inverting the tube. Prepare the fresh DNase I digestion mixture before RNA isolation.

b. OBI DNase I digestion buffer is supplied with OBI RNase-free DNase set.

c. Standard DNase buffers are not compatible with on-membrane DNase I digestion.

13. Pipet 75 µl of the DNase I digestion reaction mix directly onto the surface of HiBind® RNA resin in each column.Make sure to pipet the DNase I digestion mixture directly onto the membrane. DNase I digestion will not be complete if some of the mix stick to the wall or the O-ring of the HiBind® RNA column.

14. Incubate at room temperature(25-30°C) for 15 minutes.

15. Add 500 µl of RWC Wash buffer to HiBind RNA column and spin at 8000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

16. Place the HiBind RNA column into same collection tube. Add 500 µl RNA wash Buffer II into the HiBind RNA column and spin at 8,000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

17. Place the HiBind RNA column into same collection tube. Wash the column again by adding another 500 µl RNA wash Buffer II into the HiBind RNA column and spin at 8,000-20,000 x g for 1 minute. Discard the flow-through and re-use the collection tube.

18. Place the HiBind RNA column into the same collection tube and centrifuge at maximum speed for 2 minutes. Discard the collection tube.

19. Insert the HiBind RNA Column into a 1.5 mL Centrifuge tube(not supplied). Add 50-70 µl DEPC-water or RNase-Free water directly onto the center of the membrane in the HiBind RNA column. Incubate 1 minute at room temperature. Centrifuge at maximum speed for 2 minutes to elute RNA.

20. Incubate the 1.5 mL centrifuge tube containing eluted RNA in a incubator preset at 65°C for 5 minutes. After the incubation, immediately place the plate on ice for 5 minutes. Store RNA at -20°C or -80 °C.

Trouble Shooting Guide

Little or no RNA eluted	less blood volume in the starting sample RNA remains on the plate Measurement of RNA Plate is overloaded	• Make sure that 2.5 ml whole blood is in the Paxgene tube • Repeat elution. • Pre-heat DEPC-water to 70°C prior to elution. • Incubate for 5 min with 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 quantity of starting material.
Clogged column	Incomplete lysis	• Mix thoroughly after addition of TRK Lysis Buffer. • Reduce amount of starting material(for tissue sample)
Degraded RNA	Rnase contamination	• Check the buffer and make sur there is no RNase contamination from buffer. • Follow protocol closely, and work quickly. • Ensure not to introduce
Problem in downstream applications	Salt carry-over during elution	• Ensure Wash Buffer II Concentrate has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • 1 X Wash Buffer II must be stored and used at room temperature. • Repeat wash with Wash Buffer II.
	Inhibitors of PCR	• Use less starting material • Prolong incubation with Buffer TRK to completely lyse cells.