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Introduction

E.Z.N.A.® X-press Blood RNA Kits are designed for isolation of total intracellular RNA from up to 1 ml of fresh, whole blood treated with any common anticoagulant such as heparin, EDTA, or acid-citrate-dextrose. One ml of blood typically yields 1–5 µg of total RNA. The procedure completely removes contaminants and enzyme inhibitors making total RNA isolation fast, convenient, and reliable. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or LiCl, are eliminated. The kit is also suitable for isolation of total RNA from cultured cells, tissues, and bacteria, and from RNA viruses.

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

Principle

The E.Z.N.A.® X-press Blood RNA Kits use the reversible binding properties of HiBind® matrix, a new silica-based material. This is combined with the speed of mini-column spin technology. Red blood cells are selectively lysed and white cells collected by centrifugation. After lysis of white blood cells under denaturing conditions that inactivate RNases, total RNA is purified in the HiBind® spin column. A specially formulated high salt buffer system allows RNA molecules greater than 200 bases to bind to the matrix. Cellular debris and other contaminants (such as hemoglobin) are effectively washed away and high quality RNA is finally eluted in DEPC-treated sterile water.

Storage

E.Z.N.A.® X-press Blood RNA Kits should be stored at room temperature. During shipment crystals may form in the MRC Lysis Buffer. Warm to 37°C to dissolve. All kit components are guaranteed for at least 24 months from date of purchase

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

Kit Contents

E.Z.N.A.® Xpress Blood RNA Kits	5 Preps	50 Preps	200 Preps
Product Number	R6523-00	R6523-01	R6523-02
HiBind® RNA MicroElute Columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer ERL, 10 X Concentrate	5 ml	50 ml	3 X 50 ml
MRC Lysis Buffer	5 ml	40 ml	150 ml
RNA-Solv Reagent	5 ml	60 ml	220 ml
RWC Wash Buffer	5 ml	45 ml	220 ml
RWB Wash Buffer	4 ml	20 ml	50 ml
DEPC-ddH_2O	1.5 ml	5 ml	20 ml
Instruction Manual	1	1	1

Before Starting

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.

IMPORTANT	Dilute **10 x Buffer ERL** with sterile deionized water as follows. **R6523-00** Add 45 ml deionized water. **R6523-01** Add 450ml deionized water. **R6523-02** Add 450ml deionized water/bottle
	Dilute **RWB Wash Buffer** with **absolute ethanol** as follows. **R6523-00** Add 20 ml ethanol per bottle **R6523-01** Add 80 ml ethanol per bottle **R6523-02** Add 200 ml ethanol per bottle

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Change gloves frequently. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- During the procedure work carefully but quickly. Samples may be stored at -20°C following lysis of white blood cells with RNA-Solv Reagent.

E.Z.N.A.® XPress Blood RNA Protocol

1. **Transfer 100 µl Whole Blood into 1.5ml microcentrifuge tube and add 350 µl MRC Lysis Buffer.** Vortex at maxi speed for 30 seconds.

2. Incubate at 65oC for 10 minutes.

3. **Centrifuge at 13,000 x g for 5 minutes at room temperature.** Transfer the supernatant into a new tube.

4. **Add 0.5 volume of absolute ethanol to the lysate.** Vortex to mix well.

5. **Apply the mixture to a HiBind® RNA column assembled in a 2 ml collection tube (supplied).** Centrifuge at 10,000 x g for 30 seconds. Discard flow-through and proceed to next step.

   (Optional) On-membrane DNase Digestion: To perform on-membrane DNase digestion, follow the DNase I digestion protocol on page 7.

6. **Add 500 µl RWC Wash Buffer into the HiBind RNA spin column.** Centrifuge at >10,000 x g for 30 seconds at room temperature. Discard the 2 ml collection tube and flow-through liquid.

7. **Place column in a new 2ml collection tube (supplied), and add 500 µl RWB Wash Buffer diluted with ethanol.** Centrifuge at ≥10,000 x g for 30 seconds and discard flow-through. Re-use the collection tube for next step.
   Note: RWB Wash Buffer must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

8. **Add another 500 µl of RWB Wash Buffer to the HiBind RNA column. Centrifuge at ≥10,000 x g at room temperature. Discard flow-through.** Then with the collection tube empty, centrifuge the spin cartridge for **3 minutes at full speed** (≥14,000 x g) to *completely* dry the HiBind® matrix.

9. **ELUTION OF RNA:** Transfer the column to a new 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 15-50µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge 1 min at maximum speed.

E.Z.N.A.® XPress Blood RNA Protocol II

1. **To 1 volume of whole fresh blood (maximum of 1.5 ml) add 5 volumes of Buffer ERL (diluted).** For example add 5 ml Buffer ERL to 1 ml blood. Mix by vortexing.
   Note: Buffer ERL is supplied as a 10 x concentrate and must be diluted with sterile deionized water before use. Refer to Page 4 or label on bottle for directions.

2. Incubate for 15 min on ice, mixing by brief vortexing twice. Lysis of red blood cells is indicated when the solution becomes translucent. For blood samples from individuals with an elevated hematocrit or ECR, extend incubation time to 20 min.

3. Pellet leukocytes by centrifuging at 400 x g for 10 min at 4ºC. Completely remove and discard the supernatant containing lysed red blood cells. If a refrigerated centrifuge is not available, centrifuge at room temperature, but quickly complete Step 4 below.

4. Wash the white blood cell pellet with 2 volumes of Buffer ERL per volume of whole blood used in Step 1. Thoroughly vortex to resuspend cells.
   Tip: If you used 1 ml of whole blood, wash with 2 ml of Buffer ERL.

5. Centrifuge at 400 x g for 10 min at 4ºC. Again, completely remove and discard the supernatant. If a refrigerated centrifuge is not available, centrifuge at room temperature, but quickly complete Step 6 below.

6. Vortex at maxi speed for 30 seconds to resuspend the WBC pellet. **Add 1ml RNA-Solv Reagent and pipetting up and down 10-15 times to disrupt and homogenize the white blood cells.**

7. Incubate at room temperature for 5 minutes.

8. **Add 0.2 mL of chloroform.** Cap sample tubes securely and shake vigorously for 15 seconds. Incubate on ice for 10 minutes.

9. **Centrifuge at 12,000 x g for 15 minutes at 4°C.** The mixture separates into a lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA remains entirely in the aqueous phase.

10. **Transfer no more than 80% of the aqueous phase to a fresh tube and add 1/3 volume of absolute ethanol (~96-100%, room temperature)**. Vortex at maximum speed for 15 seconds.

    Note: All of the centrifugation step below should be carried out at room temperature.

11. **Apply the mixture from step 10 onto HiBind® RNA column assembled in a 2 ml collection tube (supplied).** *Centrifuge at 10,000 x g for 30 seconds at room temperature*. Discard flow-through and reuse the collection tube.

(Optional) On-membrane DNase Digestion: To perform on-membrane DNase digestion, follow the DNase I digestion protocol on page 7.

12. **Place column in the new 2 ml collection tube**, **and add 500 µl RWB Wash Buffer diluted with absolute ethanol.** Centrifuge as above and discard flow-through. Reuse the collection tube in next step .

    Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

13. **Wash column with a second 500 µl of RWB Wash Buffer**. Centrifuge and discard flow-through. Then with the collection tube empty, centrifuge the spin cartridge for **2 min at full speed** (≥13,000 x g) to completely dry the HiBind® matrix.

14. **Elution of RNA.** Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 30-50 µl of DEPC water (supplied with kit). Make sure to add water directly onto column matrix. let the column sit at room temperature for 2 minutes and centrifuge for 1 min at full speed. A second elution may be necessary if the expected yield of RNA >50 µg.

Optional: DNase I Digestion Protocol

Omega Bio-tek, Inc.'s RNase-Free DNase Set (product no. E1091), provides efficient on-column digestion of DNA during RNA Isolation.

1. **For each HiBind® RNA column, prepare the DNase I stock solution as follows:**

E.Z.N.A.™ DNase I Digestion Buffer	73.5 µl
RNase-free DNase I (20 Kunitz/µl)	1.5 µl
Total Volume	75 µl

2. **Add 300 µl RWC Wash Buffer directly onto the spin column.** Centrifuge at 10,000 x g for 30 seconds and discard the flow-through.

3. **Pipet 75µl of the DNase I stock solution directly onto the surface of the HiBind® RNA resin in each column**. Make sure to pipet the stock solution directly onto the membrane. DNase I Digestion will not go through completion if some of the stock solution remains stuck to the wall or the o-ring of the HiBind® RNA column.

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

5. **Add 500 µl of RWC Wash Buffer to the column.** Place the column on a bench top for 2 minutes. Centrifuge at 10,000 x g for 30 seconds and discard flow-through.

6. **Add 500µl of RWB Wash Buffer diluted with absolute ethanol.** Centrifuge at 10,000 x g for 30 seconds and discard flow-through.

7. **Add another 500µl of RWB Wash Buffer diluted to the column. Centrifuge at 10,000 x g for 30 seconds and discard flow-through** and re-use the collection tube.

8. **With the empty collection tube centrifuge the HiBind® matrix for 2 minutes at maximum speed to completely dry the HiBind® matrix.**

9. **Place the column in a clean 1.5 ml microcentrifuge tube** (not supplied), **and add 15-50 µl of DEPC-treated water** (supplied). Make sure to add water directly onto the HiBind® matrix. **Let it sit for 1 minute, and then centrifuge for 2 minutes at 10,000 x g to elute the RNA.** A second elution may be necessary if the expected yield of RNA > 30µg.

Troubleshooting Tips

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 70° C prior to elution. • Incubate column for 5 min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting material.
Clogged column	Incomplete lysis	• Mix thoroughly after addition of NTL Lysis Buffer.. • Increase centrifugation time. • Reduce amount of starting material
Degraded RNA	Source	• Do not freeze blood • Do not store blood samples for more than a few hours • Follow protocol closely, and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	• Ensure Wash Buffer II has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • 1 X Wash Buffer II must be stored at room temperature. • Repeat wash with Wash Buffer II.
	Inhibitors of PCR	• Use less starting material • Prolong incubation with Buffer ERL to completely lyse erythrocytes
DNA contamination		• Digest with RNase-free DNase and inactivate at 75°C for 5 min.