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Introduction

E.Z.N.A.® Blood RNA Kit is designed for the 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. 1 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 the isolation of total RNA from cultures cells, tissues, 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.® Blood RNA Kit uses 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 on the HiBind® spin column. A specifically 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 and stability

All components except RNA-Solv Reagent in E.Z.N.A.® Blood RNA Kits should be stored 22-25ºC. RNA-Solv® Reagent should be store at 4ºC for long term storage. All E.Z.N.A.™ Total RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as above.

*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

Product Number	R6814-00C	R6814-01C	R6814-02C
Purification times	5	50	200
HiBind™ RNA MicroElute column	5	50	200
2 ml Collection Tubes	10	100	400
Buffer ERL, 10 x Concentrate	5 ml	50 ml	3 x 50 ml
RNA-Solv Reagent	5 ml	60 ml	220 ml
RWC Wash Buffer	5 ml	45 ml	4 x 45 ml
RNA Wash Buffer II	2 ml	12 ml	4 x 12 ml
DEPC water	1.5 ml	10 ml	40 ml
Instruction Manual	1	1	1

Important Notes

Harvesting and Storage of Blood

E.Z.N.A.® Blood RNA Kit is designed for purification of total RNA from up to 1 ml **fresh** whole blood. The system is not limited by RNA binding capacity of HiBind® RNA columns (which can bind up to 100 µg RNA), but by lysis volume. Due to the abundance of erythrocytes and proteins, greater than 1 ml whole blood will significantly lower RNA quality. The relatively low RNA content of leukocytes means that the maximum binding capacity of HiBind® RNA columns can not be reached.

Samples should be collected in the presence of an anticoagulant (preferably acid-citrate-dextrose) and processed within a few hours. Minimize storage prior RNA isolation as leukocyte transcripts generally have variable stabilities.

Avoid freezing blood samples at all costs.
The E.Z.N.A.® Blood RNA procedure involves erythrocyte lysis and removal which can not be accomplished with frozen blood. For such samples we recommend the modified protocol (see page 7). Note that only 150 µl frozen blood can be used with the modified procedure.

Modified Protocols

E.Z.N.A.® Blood RNA Kit may also be used for the isolation of total RNA from cultured cells, tissues, bacteria and acellular body fluids. In addition, RNA from enzymatic reactions, such as *in vitro* transcription, can be purified with the system. Please call our Technical Staff for these additional protocols. (The E.Z.N.A.®Total RNA Kit, **product # R6634**, is recommended for isolation of total RNA from cultured cells, tissues and bacteria.)

Before Starting

IMPORTANT

1 **Buffer ERL** is supplied as a 10 x concentrate and must be diluted with sterile deionized water as follows.

R6814-00C	Add 45 ml deionized water.
R6814-01C R6814-02C	Empty contents of each bottle supplied into an appropriately sized vessel and add 450 ml deionized water per bottle of Buffer ERL.

2 **RNA Wash Buffer II Concentrate** must be diluted with absolute ethanol(~96-100%) before use.

R6814-00C	Add 8 ml absolute ethanol
R6814-01C	Add 48 ml absolute ethanol
R6814-02C	Add 48 ml absolute ethanol to each bottle

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all materials required before starting to minimize RNA degradation.

- 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 -70ºC following lysis of white blood cells with RNA-Solv Reagent.

E.Z.N.A.® Blood RNA Mini Kit Protocol

Materials supplied by user:

- 70% ethanol in DEPC-treated sterile distilled water
- Sterile RNase-free pipette tips and microcentrifuge tubes
- Tubes for erythrocyte lysis (1 ml-15 ml depending on sample size)
- Table top microcentrifuge at room temperature.
- Centrifuge with swinging-bucket rotor for 15 ml centrifuge tubes.
- Disposable latex gloves

Note: After red blood lysis and removal, all other steps must be carried out at room temperature. Work quickly, but carefully.

Procedure:

1. **To 1 volume of whole fresh blood (maximum of 1.5 ml) add 5 volumes of 1 x Buffer ERL**. 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. Blood samples from individuals with an elevated hematocrit or ECR, extend incubation time to 20 min.

3. **Pellet leukocytes by centrifuging at 450 x g for 10 min at 4°C.** Completely remove and discard the supernatant containing lysed red blood cells.

4. **Wash the white blood cell pellet with 2 volumes of 1 x 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 450 x g for 10 min at 4°C.** Completely remove and discard the supernatant.

6. Add 100µl of DEPC Water and vortex to re-suspend the white blood cells.

7. **Add 1 ml RNA-Solv Reagent and vortex thoroughly to mix.** Samples may safely be stored at -70°C after addition of RNA-Solv Reagent.

8. Homogenize the lysate by pipetting up and down for 5-10 times. Incubate at room temperature for 3 minutes.

9. **Add 0.2 mL of chloroform per 1 mL of RNA-*Solv*® Reagent.** Cap sample tubes securely and vortex vigorously for 15 seconds. Incubate on ice for 10 minutes. *This step is critical - do not change it.*

10. **Centrifuge at 12,000 x g for 15 minutes 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.

11. Transfer no more than 80% of the aqueous phase to a fresh tube, and **add an equal volume of 70% ethanol and vortex to mix well.**

12. **Apply the entire sample to a HiBind® RNA microElute column assembled in a 2 ml collection tube (supplied).** The maximum capacity of the HiBind™ RNA spin cartridge is 800µl. (Larger volumes can be loaded successively.) Centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and proceed to step 13.

13. **Wash column with RWC Wash Buffer by pipetting 500 µl directly into the spin column.** Centrifuge as above and **discard the 2 ml collecting tube**.

14. **Place column in a clean 2ml collection tube (supplied), and add 500 µl RNA Wash Buffer II diluted with ethanol.** Centrifuge as above and discard flow-through.

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

15. Then with the collection tube empty, centrifuge the column for **2 min at** 10,000 x g at room temperature to completely dry the HiBind® matrix.

16. **Elution of RNA.** Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 15-30 µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge at 10,000 x g for 1 minutes.

E.Z.N.A.® X-Press Protocol for Blood Samples

This protocol is designed for fast isolation of Total RNA from 1-150ul fresh or frozen Blood.

1. **Pipette 100-200 µl of blood into a sterile microcentrifuge tube**.

2. **Add 1 ml RNA-Solv Reagent and vortex thoroughly to mix.** Incubate at room temperature for 3 minutes.

3. **Add 0.2 mL of chloroform per 1 mL of RNA-*Solv*® Reagent.** Cap sample tubes securely and vortex vigorously for 15 seconds. Incubate on ice for 10 minutes. *This step is critical - do not change it*.

4. **Centrifuge at 12,000 x g for 15 minutes 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.

5. Transfer no more than 80% of the aqueous phase to a fresh tube, and **add an equal volume of 70% ethanol and vortex to mix well.**

6. Proceed to step 12, (page 6) of main protocol (addition of sample to RNA HiBind®column/collection tube assembly).

Quantitation and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm in a spectrophotometer. 1 O.D. unit measured at 260 nm corresponds to 40 µg of RNA per ml. The ratio of A_{260}/A_{280} of pure nucleic acids is 2.0, while for pure protein it is approximately 0.6. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. (Phenol has an absorbance maximum at 275 nm and can interfere with spectrophotometric analysis of DNA or RNA. However, the E.Z.N.A.® Total RNA Kit eliminates the use of phenol and avoids this problem.) Store RNA samples at -70ºC in water. Under such conditions RNA prepared with the E.Z.N.A. system is stable for more than a year.

RNA Quality

It is highly recommended that RNA quality be determined prior to all analyses. The quality of RNA can be assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Two sharp bands should appear on the gel. These are the 28S and 18S (23S and 16S for bacteria) ribosomal RNA bands. If these band smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling, or storage. Although RNA molecules less than 200 bases in length do not efficiently bind the HiBind matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Trouble Shooting Guide

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 65º C prior to elution. • Incubate column for 10 min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting material.
Clogged column	Incomplete homogenization	• Completely homogenize sample. • Increase centrifugation time. • Reduce amount of starting material
Degraded RNA	Source	• Freeze starting material quickly in liquid nitrogen. • Do not store tissue culture cells prior to extraction unless they are lysed first. • 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 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.
DNA contamination		• Digest with RNase-free DNase and inactivate at 75ºC for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	• DEPC water is acidic and can dramatically lower Abs260 values. Use TE buffer to dilute RNA prior to spectrophotometric analysis.