Contents

Revised January 2008

Introduction

The E.Z.N.A.® Viral RNA Kit is designed for isolation of Viral RNA from cell free fluids such as plasma, serum, urine, and cell culture supernatants. The procedure completely removes contaminants and enzyme inhibitors, making viral RNA isolation fast, convenient, and reliable. This kit has been tested for isolating viral nucleic acids from hepatitis A, C, and HIV. The kit is also suitable for isolation of total RNA from cultured cells, tissues, and bacteria.RNA purified using the E.Z.N.A.® Viral RNA method is ready for applications such as RT-PCR*.

The E.Z.N.A.® Viral RNA Kit uses reversible binding properties of HiBind® matrix, a new silica-based, time saving spin technology material. Combined with the speed of mini-column spin technology or vacuum manifold, multiple samples can be processed at the same time. The sample is lysed first under highly denaturing buffer conditions so that RNases will be inactivated, and the intact viral RNA is protected from degrading. After adjusting the buffer condition, the samples are loaded to the HiBind® RNA column. With a brief centrifugation or vacuum, the samples pass through the column and the viral RNA binds to the Hibind® matrix. After two washing steps, purified viral RNA will be eluted with RNase-free water.

Note: The E.Z.N.A.® Viral RNA Kit is not designed to separate viral RNA from cellular RNA and DNA. It will purify both in parallel if they present in the sample. Cell free body fluids are recommended.

Storage and stability

All components in the E.Z.N.A.® Viral RNA Kit should be stored at room temperature except QVL Lysis buffer. QVL Lysis Buffer/Carrier RNA must be stored at 2-8ºC . During shipping and storage, crystals may form in the QVL Lysis Buffer, simply warm to 37ºC to dissolve. All kit components are guaranteed for at least 12 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

Product Number	R6874-00	R6874-01	R6874-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ RNA Mini Columns	5	50	200
2 ml Collection Tubes	15	150	600
QVL Lysis Buffer	5 ml	30 ml	120 ml
RWA Wash Buffer	3.8 ml	19 ml	76 ml
RWB Wash Buffer	2 ml	12 ml	40 ml
Carrier RNA	50 µg	310 µg	1.2 mg
DEPC Water	1.5 ml	10 ml	30 ml
Instruction Manual	1	1	1

QVL Lysis Buffer contains a chaotropic salt. Use gloves and protective eyeware when handling this solution.

Important Notes

- 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.
- Carefully apply the sample or solution to the HiBind RNA column. Avoid touching the membrane with the pipet tip.
- Sample volume: HiBind® RNA spin column can bind any RNA greater than 200nt. Yield will depend on the sample sources and conditions. The protocol is optimized for use with 140 µl samples. Smaller samples should be adjusted to 140 µl with PBS or DEPC water; lower titer samples should be concentrated to 140 µl before processing. For samples larger than 140 µl, the amount of QVL Lysis buffer and other reagents added to the sample before loading must be increased proportionally.

Before Starting

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.

- **Dilute RWB Wash Buffer Concentrate** with absolute ethanol before use.

R6874-00	Add 8 ml absolute ethanol (96-100%) to bottle
R6874-01	Add 48 ml absolute ethanol (96-100%) to bottle
R6874-02	Add 160 ml absolute ethanol (96-100%) to bottle

- **Dilute RWA Wash Buffer Concentrate** with absolute ethanol before use.

R6874-00	Add 5 ml absolute ethanol (96-100%) to bottle
R6874-01	Add 25 ml absolute ethanol (96-100%) to bottle
R6874-02	Add 100 ml absolute ethanol (96-100%) to bottle

- Add 50 µL(5 preps), 310 µL (50 preps), 1.2ml (200 preps) DEPC Water to the tube containing lyophilized Carrier RNA to obtain a solution 1µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20oC. Do not freeze-thaw the aliquots of carrier RNA more than 3 times.

Materials to Be Provided by User

- Absolute ethanol (96-100%)
- Sterile RNase-free pipette tips and microcentrifuge tubes
- Table top microcentrifuge at room temperature.
- 2-mercaptoethanol
- Disposable latex gloves
- (Optional)Vacuum manifold with standard leur adaptor

E.Z.N.A.® Viral RNA Spin Protocol

Note: Equilibrate samples and QVL Lysis buffer to room temperature before beginning. All steps must be carried out at room temperature. Work quickly, but carefully.

1. **Add 560 µl QVL Lysis buffer, 5.6 µl Carrier RNA and 10 µl 2-mercaptoethanol into a 1.5 ml micro-centrifuge tube.**
   Note: *QVL Lysis Buffer, Carrier RNA and 2-mercaptoethanol can be premixed together*. The premixed lysis buffer can stored at 2-8oC for 1 week. Increase the amount of Lysis Buffer proportionally if the sample volume is larger than 140 µl.

2. **Pipet 140 µl plasma, cell free body fluid or urine into the micro-centrifuge tube containing Lysis Buffer**. Mix throughly by vortexing at maxi speed for 30 seconds.

3. Incubate at room temperature for 5-10 minutes. Briefly spin to collect any liquid from lid.

4. **Add 560 µl of absolute ethanol (room temperature, 96-100%) to the sample, mix throughly by vortexing at maxi speed for 30 seconds**.

5. **Apply the 650 µl of the mixture (including any precipitate) to a HiBind® RNA column assembled in a 2 ml collection tube (supplied)**. The maximum capacity of the HiBind™ RNA column is 800 µl. During the procedure, work carefully but quickly. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through.

6. Repeat step 5 until all the lysate has been loaded into the column and passed through the column.

7. **Place the column into a new 2 ml collection tube (from step 6)**, **and add 500 µl RWA Wash Buffer diluted with ethanol**. Centrifuge as above and discard flow-through.

   Note: RWA Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle or 'before starting' on page 4 for directions.

8. Place the column into a new 2 ml collection tube and add **500ul RWB Wash Buffer diluted with ethanol. Centrifuge as above and discard the flow-through.**

   Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle or 'before starting' on page 4 for directions.

9. Place the column back into the collection tube, centrifuge the empty column at full speed(no more than 12,000 x g) for 3 min to completely dry the HiBind™ matrix.

10. Transfer the column to a clean 1.5 ml microfuge tube (not supplied) and add 30-50 µl DEPC water (supplied with kit) directly onto column matrix. Allow the column to incubate for 3 to 5 min at room temperature. Centrifuge at 10,000 x g for 1 min to elute RNA. Store Purified RNA at -70º C.

E.Z.N.A.® Viral RNA Vacuum Protocol

Note: Equilibrate samples and QVL Lysis buffer to room temperature before beginning. All steps must be carried out at room temperature. Work quickly but carefully. Become familiar with the manifold by reading the instructions for the manifold before starting vacuum protocol.

1. Prepare the lysate by following step 1-4 of Spin Protocol on page 5.

2. Assemble the HiBind® RNA column into the vacuum manifold by plugging the column into the leur adaptor of the manifold.

3. Load the 650 µl of the lysate (including any precipitation) to the column. Switch on vacuum source to draw the sample through the column. The maximum capacity of the HiBind™ RNA column is 800 µl. During the procedure, work carefully but quickly. Load the lysate successive onto the column and draw all of the lysate through the column. Turn off the vacuum source.

   Note: If for any reason the solution has trouble passing through the column with vacuuming, take the column and assemble it into a 2 ml collection tube, spin at 10,000 rpm for 5 minutes or until all the sample passes through the column. Continue with step 7 in centrifugation protocol.

4. **Wash the column by adding 500 µl RWA Wash Buffer diluted with absolute thanol**, draw the solution through the column by turning on the vacuum source.

5. **Wash the column again by adding 500 µl RWB Wash Buffer** diluted with absolute ethanol, draw the wash buffer through the column by turning on the vacuum source. Turn off the vacuum source.

6. Close the cap of the column, remove it from manifold and assemble it into a new 2 ml collection tube. Centrifuge at maxi speed (no more than 14,000 x g) for 2 minute to dry the membrane completely.

7. Transfer the column to a clean 1.5 ml microfuge tube (not supplied) and add 20-

30µl DEPC-treated water (supplied with kit) directly onto column matrix. Allow the column to incubate for 3 to 5 min at room temperature. Centrifuge at 10,000 x g for 1 min to elute RNA. Store Purified RNA at -70° C.

Quantization and Storage of RNA

To determine the concentration and purity of RNA, measure absorbency at 260 nm and 280 nm in a spectrophotometer. 1 O.D. unit measured at 260 nm corresponds to 40 µl 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 absorbency maximum at 275 nm and can interfere with spectrophotometric analysis of DNA or RNA. However, the E.Z.N.A.® RNA Isolation technology eliminates the use of phenol and avoids this problem.) Store RNA samples at -70°C in DEPC-treated water. Under such conditions RNA prepared with the E.Z.N.A. system is stable for more than a year.

Protocol for Isolation of Viral, Cellular, Bacterial, or Viral DNA from Urine:

The QVL lysis buffer can inactivate the numerous PCR inhibitors found in Urine. So this product can be used for isolation of cellular, bacterial, or viral DNA from urine for use in PCR. We recommend the use of the centrifugation protocol. Since urine contains very low number of cells, bacteria and viruses, samples often need to be concentrated to final volume of 150 µl to use spin protocol.

Troubleshooting Guide

Problem	Cause	Suggestion
Little or no RNA eluted	Carrier RNA not added to QVL Buffer or degraded	• Dissolve the carrier RNA with QVL Buffer and repeat the purification with new sample. • Avoid warm the QVL/Carrier RNA frequently.
	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 QVL Lysis Buffer. • Increase centrifugation time. • Reduce amount of starting material
Degraded RNA	Source	• Do not freeze and thaw sample more than once. • Follow protocol closely, and work quickly. • Low concentration of virus in the sample
	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.
DNA contamination		• Digest with RNase-free DNase and inactivate at 75°C for 5 min.