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Introduction

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*.

Principle

The E.Z.N.A.® Viral RNA Kits use 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

E.Z.N.A.® Viral RNA Kits are 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

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 2-8°C . During shipping and storage, crystals may form in the QVL Lysis Buffer, simply warm to 37°C 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

E.Z.N.A.® Viral RNA Kits	5 Preps	50 Preps	200 Preps
Product Number	R6874-00	R6874-01	R6874-02
Purifications	5	50	200
Components			
HiBind™ RNA Columns	5	50	200
2 ml Collection Tubes	15	150	600
QVL Lysis Buffer	5 ml	30 ml	120 ml
RNA Wash Buffer I	5 ml	40 ml	200 ml
RNA Wash Buffer II Concentrate	5 ml	12 ml	50 ml
Carrier RNA		1.5mg	6 mg
DEPC-ddH_2O	1.5 ml	10 ml	30 ml
Instruction Manual	1	1	1

Important Notes

1. Carrier RNA dissolved in QVL Buffer must be stored at 2-8° C, and it should be stable for up to 6 months. QVL/carrier RNA solution is only stable for 10 days at room temperature. DO NOT frequently warm up QVL/Carrier RNA solution. It is recommended to make aliquots of this buffer according to average usage per week. **Note: Carrier RNA has been pre-dissolved with R6874-00.**

2. 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. 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.

3. 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 150 µl samples. Smaller samples should be adjusted to 150 µl with PBS or DEPC water; lower titer samples should be concentrated to 150 µl before processing. For samples larger than 150 µl, the amount of QVL Lysis buffer and other reagents added to the sample before loading must be increased proportionally.

Before Starting

IMPORTANT

1. **Adding Carrier RNA to Buffer QVL Lysis buffer**: add 1ml QVL Buffer to the tube of lyophilized Carrier RNA. Completely dissolve Carrier RNA and transfer the mixture to the Buffer QVL buffer bottle. Mix throughly by shake few times.

2. **Wash Buffer II Concentrate** must be diluted with absolute ethanol before use.

Trial Sample (R6874-00)	Add 20 ml 100 % ethanol
R6874-01	Add 48 ml 100 % ethanol
R6874-02	Add 200 ml 100% ethanol

E.Z.N.A.® Viral RNA Spin Protocol

Materials supplied by user:
- 96-100% ethanol
- Sterile RNase-free pipette tips and microcentrifuge tubes
- Table top microcentrifuge at room temperature.
- Disposable latex gloves

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

Procedure:

1. Add 500 µl QVL Lysis buffer into a 1.5 ml microcentrifuge tube.

Note: Make sure that Carrier RNA is added to Buffer QVL according to the instructions. Increase the amount of QVL/Carrier RNA proportionally if the sample volume is larger than 180 µl .

2. Pipet 150 µl plasma, cell free body fluid, cell culture or urine into the microcentrifuge containing QVL/Carrier RNA. Mix throughly by vortexing for 30 seconds.

3. Incubate at room temperature for 5-10 minutes.

4. Spin briefly to collect any liquid from lid.

5. Add 350 µl of absolute ethanol (96-100%) to the sample, mix throughly by vortexing for 30 seconds. Centrifuge briefly to collect any liquid droplets from lid.

6. Apply the 750 µl of sample (including any precipitate) to a HiBind® RNA column assembled in a 2 ml collection tube (supplied). The maximum capacity of the HiBind™ RNA spin cartridge is 800 µ**l**. During the procedure, work carefully but quickly. (Larger volumes can be loaded successively.) Centrifuge at 10,000 x g for 15 seconds. Discard flow-through and proceed to step 7.

7. Repeat step 6 until all the lysate has been loaded on to the spin column.

8. **Wash column with Wash Buffer I by pipetting 750 µl directly into the spin column.** Centrifuge as above and **discard the 2 ml collection tube**.

9. **Place column in a clean 2ml collection tube (supplied)**, and add 500 µl Wash Buffer II diluted with ethanol. Centrifuge and discard flow-through. Reuse the collection tube in step 10.

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

10. With the collection tube empty, insert and centrifuge the spin cartridge for 1 min at full speed to completely dry the HiBind™ matrix.

11. Elution of RNA: Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 50-100 µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge 1 min at maximum speed.

No RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR or differential display) we suggest that you treat the eluted RNA with RNase-free DNase. Also for RT-PCR, use intron-spanning primers that allow easy identification of DNA-contamination. A control PCR reaction containing the RNA as template will also allow detection of DNA contamination. For designing intron-spanning primers, call our technical staff at 800-832-8896 for assistance.

E.Z.N.A.® Viral RNA Vacuum Protocol

Materials supplied by user:

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

Note: Equilibrate samples and QVL 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. **Pipette 500 µl of QVL/Carrier solution into a RNase-free microcentrifuge tube.**
Note: Add 20 µl Buffer/ß-mercaptoethanol to each 1ml QVL buffer before use.

2. Add 150 µl of sample (serum, urine, cell culture supernatant or cell-free body fluids) to the QVL/Carrier RNA solution in the microcentrifuge tube. Mix throughly by vortexing for 30 seconds.

3. Incubate at room temperature for 5-10 minutes.

4. Spin briefly to collect any liquid from lid

5. Add 350µl of absolute ethanol (96-100%) to the sample; mix throughly by vortexing for 30 seconds. Centrifuge briefly to collect any liquid droplets from lid.

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

7. Apply the 750 µl of sample (including any precipitate) to a HiBind® RNA column assembled in a 2 ml collection tube (supplied). The maximum capacity of the HiBind™ RNA spin cartridge is 800 µ**l**. During the procedure, work carefully, but quickly. (Larger volumes can be loaded successively.) Switch on the vacuum source, be sure

to leave the cap of the lid of the column open while applying vacuum. Switch off the vacuum source after all sample has been drawn through the column.
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 10,000rpm for 5 minutes or until all the sample passes through the column. Continue with step 7 in centrifugation protocol.

8. Repeat step 7 until all lysate has been loaded on to the spin column.

9. **Wash column with Wash Buffer I by pipetting 750 µl directly into the spin column.** Switch on vacuum source to draw the solution through the membrane.

10. **Wash column with Wash Buffer II by pipetting 750 µl directly into the spin column.** Switch on vacuum source to draw the solution through the membrane.

11. Close the cap of the spin column, remove it from manifold and assemble it into a new 2 ml collection tube. Centrifuge for 1 minute to dry the membrane completely.

12. Elution of RNA. Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 50-100 µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge 1 min at maximum speed.

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 water. Under such conditions RNA prepared with the E.Z.N.A. system is stable for more than a year.

Protocol for Isolation of Cellar, 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 Tips

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.
	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.