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Introduction

E-Z 96® Viral RNA Kit is designed for isolation of Viral RNA from cell free fluids such as plasma, serum, urine, and cell culture supernatant. 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, HIV. The kit is also suitable for isolation of total RNA from cultured cells, tissues, and bacteria.

RNA purified using the E-Z 96® Viral RNA method is ready for applications such as RT-PCR*.

Principle

The E-Z® 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 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 Plate. With a brief centrifugation or vacuum, the samples pass through the plate 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 96® Viral RNA Kits are not designed to separate viral RNA from cellular RNA and DNA. It will purify both in parallel if they are present in the sample. Cell free body fluids are recommended.

Storage

All components in the E-Z 96® Viral RNA Kit should be stored at room temperature except QVL Lysis buffer. QVL Lysis Buffer/Carrier RNA solution 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 96® Viral RNA Kits	4 x 96 Preps	12 x 96 Preps
Product Number	R1074-01	D1074-02
Purification	4	12
Components		
HiBind™ RNA Plates	4	12
Racked Microtubes (1.2ml)	4 x 96	12 x 96
8-Strip Microtube Caps	48 x 8	144 x 8
2 ml 96-well Collection Plates*	2	2
QVL Lysis Buffer	220 ml	700 ml
RNA Wash Buffer I	300 ml	900 ml
RNA Wash Buffer II Concentrate	2 x 50 ml	3 x 100 ml
Carrier RNA	3 mg	9 mg
DEPC-ddH_2O	40 ml	120 ml
Instruction Manual	1	1

* 2ml Deep well plates are reusable, see page 7 for instruction.

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 a maximum of 21 days at room temperature. DO NOT frequently warm up QVL/Carrier RNA solution. It is recommended that aliquots of this buffer be made according to average usage per week.

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 touch the membrane with pipet tip.

3. Sample volume: HiBind® RNA resin 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**: Dilute carrier RNA with QVL Buffer to the final concentration of 10 µl/ml. Use 1 ml QVL Buffer to 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. R1074-01 Add 200 ml 100 % ethanol R1074-02 Add 400 ml 100% ethanol
	3	Reconstitute OB Protease in 1 ml 10 mM Tris-HCl, pH 8. Vortex vial briefly prior to use.

E-Z 96® Viral RNA Protocol with Centrifugation

Materials supplied by user:

- 96-100% ethanol
- β-Mercaptoethanol
- Multichannel pipet
- RNase-free filter pipette tips
- Reagent reservoirs for multichannel pipets
- Centrifuge with suitable rotor for 96-well plate.
- Disposable latex gloves
- RNase-Free 1.2 ml 96-well plate
- Adhesive sealing film for microplate
- 2ml 96-well deep well plate

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

Procedure:

1. **Use a 1.2 ml 96-well plate to prepare sample**. Add 150 µl plasma, cell free body fluid, cell culture or urine into each well of the 96-well plate.

2. Pipet 10ul of reconstituted OB Protease solution into each well of the 96-well plate.

3. Pipet 550 µl QVL lysis buffer into the each well of the 96-well plate. Keep the microplate flat on the bench, shake vigorously back and forth for 30 seconds. Rotate the plate 90° and shake the plate for another 30 seconds.

   Note: Add 20 µl Buffer/ß-mercaptoethanol with each 1ml QVL buffer before use. Make sure that Carrier RNA is added to QVL Lysis buffer according to the instructions.

4. Seal the plate with sealing film. Incubate at room temperature for 5-10 minutes.

5. Spin at 500 x g briefly to collect any liquid.

6. Add 560µl of absolute ethanol (96-100%) to the sample, mix throughly by vortexing for 30 seconds. Centrifuge briefly to collect any liquid droplets from lid.

7. **Carefully** apply 700µl samples from step 6 (including any precipitate) to each well of the HiBind® RNA plate.

8. Seal the HiBind® RNA plate with new sealing film. Load the HiBind® RNA plate with 2ml 96-well plate into the plate holder, and place the whole assembly into the rotor bucket of the centrifuge. Spin at 5500 x g for 5 minutes at room temperature.

9. Remove the sealing film and load remaining sample from step 7 into HiBind® RNA plate. Seal the HiBind® RNA plate with new sealing film. Load the HiBind® RNA plate with 2ml 96-well plate into the plate holder, and place the whole assembly into the rotor bucket of the centrifuge. Spin at 5500 x g for 5 minutes at room temperature.

10. Remove the sealing film. Wash plate with RNA Wash Buffer I by pipetting 750 µl directly into the each well of the HiBind® RNA plate **.** Seal the plate with a new sealing film. Centrifuge at 5500 x g for 5 minutes at room temperature.

11. **Place HiBind® RNA plate on top of 2ml 96-well plate.** Remove the sealing film and add 500 µl Wash Buffer II diluted with ethanol to each well of HiBind® RNA plate. Seal the plate with a new sealing film. Centrifuge at 5500 x g for 5 minutes at room temperature.

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

12. Remove the sealing film. Add another 500ul of RNA Wash Buffer II to each well of HiBind® RNA plate. Seal the plate with a new sealing film. Centrifuge at 5500 x g for 10 minutes at room temperature.
Note: It is very important to dry the HiBind® RNA plate before the elution because the residual ethanol might interfere with downstream applications.

13. Elution of RNA: Remove the sealing film and place the HiBind® RNA plate onto the microtube rack containing 1.2ml microtubes (supplied with kit).

14. Add 50-75 µl of DEPC-treated water to each well, and seal the HiBind® RNA plate with new sealing film(supplied with kit). Make sure to add water directly onto RNA matrix. Incubate for 1 minute at room temperature. Centrifuge at 5500 x g for 5 minutes at room temperature to elute RNA.

15. Use the 8-strip caps to seal the micrtubes for storage.

E-Z 96® Viral RNA Vacuum Protocol

Materials supplied by user:

- 96-100% ethanol
- β-Mercaptoethanol
- Multichannel pipets
- RNase-free filter pipette tips
- Reagent reservoirs for multichannel pipets
- Vacuum Manifold (Product# Vac-03)
- Vacuum source capable of generating a vacuum pressure of -900 mbar
- Disposable latex gloves
- 800 µl or 1.2 ml 96-well plate
- 2ml deep 96-well plate

Note: Equilibrate samples and QVL buffer to room temperature before starting. 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 this vacuum protocol.

1. **Prepare the vacuum manifold according to the manufacturer's instruction. Place the 2ml 96-well deep well plate (supplied) or a waste collection tray inside the vacuum manifold base. Place the top plate squarely over the base. Place the HiBind® RNA plate on the top plate, making sure that the HiBind® RNA plate is seated tightly on the rubber ring of the top plate. Connect the Vacuum manifold to the vacuum source. Keep the vacuum switch off.**

2. **Use a 1.2 ml 96-well plate to prepare sample.** Pipet 10ul of reconstituted OB Protease solution into each well of the 96-well plate.

3. Add 150 µl plasma, cell free body fluid, cell culture or urine into each well of the 96-well plate.

4. Pipet 550 µl QVL lysis Buffer into the each well of the 96-well plate. Keep the microplate flat on the bench; shake vigorously back and forth for 30 seconds. Rotate the plate 90° and shake the plate for another 30 seconds.

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

6. Add 560µl of absolute ethanol (96-100%) to the sample, mix throughly by pipetting up and down 3-4 times.

7. Apply 700µl sample from step 5 into wells of HiBind® RNA plate, switch on the vacuum source. Apply vacuum until all sample contents pass through the membrane.

8. Apply rest of sample from step 5 into wells of HiBind® RNA plate, switch on the vacuum source. Apply vacuum until all sample contents pass through the membrane. Switch off the vacuum, and ventilate the manifold.

9. Lift the top plate carrying the HiBind® RNA plate from the base, and empty the waste from 2ml 96-well plate or the waste tray. Reassemble the manifold.

10. Wash plate with RNA Wash Buffer I by pipetting 750 µl directly into the each well of the HiBind® RNA plate. Apply the vacuum until transfer is

complete. Switch off the vacuum, and ventilate the manifold.

11. Add 500 µl RNA Wash Buffer II to each well of the of HiBind® RNA plate, and apply the vacuum until transfer is complete. Switch off the vacuum, and ventilate the manifold.
Note: Wash Buffer II Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

12. Add 500 µl RNA Wash Buffer II to each well of the of HiBind® RNA plate, and apply the vacuum until transfer is complete. Switch off the vacuum, and ventilate the manifold.

13. Remove the HiBind® RNA plate from top plate of vacuum manifold, and strike the bottle of the HiBind® RNA plate on a stack of paper towels. Repeat for few times until no liquid is released onto the paper towels.

14. Place the HiBind® RNA plate back to the top plate of the manifold. Apply maximum vacuum for 15 minutes. Turn off the vacuum source and ventilate the manifold.

15. Replace the 2ml deep well plate or waste collection tray with a microtube rack containing the 1.2ml microtubes (supplied). Reassemble the manifold. Place the HiBind® RNA plate on top plate of manifold.

16. Elution RNA: Add 60-75 µl of DEPC-treated water to each well, and seal the HiBind® RNA plate with new sealing film(supplied with kit). Make sure to add water directly onto RNA matrix. Incubate for 1 minute at room temperature. Switch on the vacuum source for 5 minutes. Switch off the vacuum, and ventilate the manifold.

17. Use the 8-strip caps to seal the micrtubes for storage.

Quantitation 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 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 absorbency maximum at 275 nm and can interfere with spectrophotometric analysis of DNA or RNA. However, the HiBind® RNA technology eliminates the use of phenol and avoids this problem.) Store RNA samples at -70°C in water. Under such conditions RNA 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.

Clean the 2ml deep well plates:
Two 2ml deep well plate are supplied with each kit. If extra plates are needed, please call our customer service department for ordering information. To reuse the deep well plates, rinse them throughly with tap water, incubate overnight in 0.2M NaOH/1mM EDTA, rinse with distilled water and dry by air.

Troubleshooting Tips

Problem	Cause	Suggestion
Little or no RNA eluted	Carrier RNA not added to QVL Buffer or degraded RNA remains on the plate	• Dissolve the carrier RNA with QVL Lysis Buffer and repeat the purification with new sample. • Avoid warming the QVL/Carrier RNA frequently. • Repeat elution. • make sure to use the vacuum source capable of generating vacuum pressure of -800 to -900mbar. • Incubate for 5 min with water prior to elution
	Plate is overloaded	• Reduce quantity of starting material.
Abnormal OD reading on A260/A280	DEPC residue remains in DEPC-water	• use different RNase-free water. • Remove DEPC by Autoclave • Use 10mM Tris-HCl, not the DEPC water to dilute the sample before measuring purity
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 QVL to completely lyse cells
DNA contamination		• Digest with RNase-free DNase and inactivate at 75°C for 5 min.