## Contents




**Revised June 2002**




## Introduction

The 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, and 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*.

The E-Z 96® 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 E-Z 96 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:** The E-Z 96® 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 96® Viral RNA Kit, except of Carrier RNA should be stored at room temperature. Store Carrier RNA at -20°C. Store QVL Lysis Buffer/Carrier RNA Mixture 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 | R1074-01 | D1074-02 |
|---|---|---|
| Purification | 4 x 96 Preps | 12 x 96 Preps |
| E-Z 96™ RNA Plates | 4 | 12 |
| 1.2ml collection Plates | 4 x 96 | 12 x 96 |
| 8-Strip Microtube Caps | 100 x 8 | 150 x 8 |
| 2 ml 96-well Collection Plates* | 2 | 2 |
| QVL Lysis Buffer | 2 x 120 ml | 6 x 120 ml |
| Buffer VHB | 110 ml | 2 x 220 ml |
| RNA Wash Buffer II | 2 x 50 ml | 6 x 50 ml |
| Carrier RNA | 2 x 1.2 mg | 6 x 1.2 mg |
| DEPC-dd$H_2O$ | 100 ml | 2 x 100 ml |
| Instruction Manual | 1 | 1 |

**Note:** 96-Well Collection Plates (2ml) can be used to collect flow-through from the E-Z 96® RNA plate. They are designed for repeated use. Wash the plates thoroughly in tap water after each use. Incubate 5 minutes at room temperature in 0.5M HCl. Rinse with distilled water. Used plates can also be autoclaved after washing. 2 96-well collection plates are supplied with each kit. If extra plates are needed, please call our customer service department for ordering information.

## 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 E-Z 96™ RNA Plate. Avoid touching the membrane with the pipet tip.
- Sample volume: Each Well of E-Z 96™ RNA Plate 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

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.

- **Dissolve Carrier RNA to Buffer QVL Lysis Buffer** as following. Dilute carrier RNA with QVL Lysis Buffer to the final concentration of 10 µg/ml. Use 1 ml QVL Lysis Buffer to completely dissolve Carrier RNA and transfer the mixture to the Buffer QVL Lysis buffer bottle. Mix throughly by shake few times.

  **Carrier RNA dissolved in QVL Lysis Buffer must be stored at 2-8° C**, and it should be stable for up to 6 months. DO NOT frequently warm up QVL/Carrier RNA solution. It is recommended to make aliquots of this buffer according to average usage per week.

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

| | |
|---|---|
| **R1074-01** | Add 200 ml absolute ethanol (96-100%) to each bottle |
| **R1074-02** | Add 200 ml absolute ethanol (96-100%) to each bottle |

**Store the diluted RNA Wash Buffer II at room temperature.**

- **Dilute Buffer VHB** with absolute ethanol before use.

| | |
|---|---|
| **R1074-01** | Add 140 ml absolute ethanol (96-100%) to each bottle |
| **R1074-02** | Add 280 ml absolute ethanol (96-100%) to each bottle |

**Store the diluted RNA Wash Buffer II at room temperature.**

## Materials to Be Provided by User

- 96-100% ethanol and ß-Mercaptoethanol
- Multichannel pipet and Reagent reservoires
- Centrifuge with suitable rotor for 96-well plate.
- Disposable latex gloves and Rnase-free filter tips
- RNase-Free 1.2 ml 96-well plate and 2ml 96-well plate
- Adhesive sealing film for microplate
- **(Optional for vacuum protocol) Vacuum Modified**

## E-Z 96® Viral RNA Spin Protocol

**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.

1. **Use a 1.2 ml 96-well plate to prepare sample. Add 100 µl plasma, cell free body fluid, cell culture or urine into each well of the 96-well plate.**

2. **Pipet 500 µl QVL lysis buffer/Carrier RNA into the each well of the 96-well plate.** Seal the plate with 8-Strip Microtube Caps. 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. Incubate at room temperature for 5-10 minutes.
   Note: Add 20 µl Buffer/ß-mercaptoethanol with each 1 ml QVL Lysis buffer/Carrier RNA before use. Make sure that Carrier RNA is added to QVL Lysis buffer according to the instructions.

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

4. Remove the 8-strip microtube caps. **Add 350µl of absolute ethanol (room temperature, 96-100%) to the sample.**

5. Seal the plate with 8-strip microtube caps. 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. Centrifuge briefly to collect any liquid droplets from lid.

6. **Carefully** apply the entire samples from step 5 to each well of the E-Z 96® RNA plate.

7. Seal the E-Z 96® RNA plate with new sealing film. Load the E-Z 96® 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 4000 x g for 5 minutes at room temperature. Discard the flow-through and re-use 2 ml collection Plate.

8. Remove the sealing film. **Place E-Z 96® RNA plate back onto 2ml 96-well collection plate and add 500 µl Buffer VHB diluted with ethanol to each well of the E-Z 96® RNA plate .** Seal the plate with a new sealing film. Centrifuge at 4000 x g for 5 minutes at room temperature.

9. Discard the flow-through and place E-Z 96® RNA plate on top of the 96-well plate. **Remove the sealing film and add 500 µl RNA Wash Buffer II diluted with ethanol to each well of E-Z 96® RNA plate.** Seal the plate with a new sealing film. Centrifuge at 4000 x g for 5 minutes at room temperature.

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



10. Discard the flow-through and place E-Z 96® RNA plate on top of the 96-well plate. Remove the sealing film. **Add another 500ul of RNA Wash Buffer II to each well of E-Z 96® RNA plate.** Seal the plate with a new sealing film. Centrifuge at 4000 x g for 10 minutes at room temperature.

    Note: It is very important to dry the E-Z 96® RNA plate before the elution because the residual ethanol might interfere with downstream applications.

11. Elution of RNA: Remove the sealing film and **place the E-Z 96® RNA plate onto a new 96 well plate (Not supplied with kit).**

12. **Add 50-70µl of DEPC-treated water to each well, and seal the E-Z 96® RNA plate with new sealing film(supplied with kit).** Make sure to add water directly onto RNA matrix.

13. Incubate for 1 minute at room temperature. Centrifuge at 4000 x g for 5 minutes at room temperature to elute RNA.

14. Remove the sealing film. Repeat step 12 and 13 for second elution.

## E-Z 96® Viral RNA Vacuum Protocol

**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:** Place the waste 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. **Prepare the lysate and binding condition of sample by following spin protocol step 1-5 on page 5.**

3. Apply the entire Sample/QVL/ethanol mixture (including any precipitation) into wells of E-Z 96® RNA plate, switch on the vacuum source. Apply vacuum until all sample contents pass through the Plate.

4. Wash plate with Buffer VHB by pipetting 500 µl directly into the each well of the **E-Z 96®** RNA plate. Apply the vacuum until transfer is complete. Switch off the vacuum.
5. Add 500 µl RNA Wash Buffer II to each well of the of **E-Z 96®** RNA plate, and apply the vacuum until transfer is complete. Switch off the vacuum

.

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

6. Repeat step 5 for second wash with RNA Wash Buffer II.

7. Remove the **E-Z 96**® 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.

8. Place the **E-Z 96**® RNA plate back to the top plate of the manifold. Apply vacuum for 15 minutes. Turn off the vacuum source and ventilate the manifold.

9. Replace the 2ml deep well plate with a new 96 well plate(Not supplied with kit). Reassemble the manifold. Place the HiBind® RNA plate on top plate of manifold.

10. Elution RNA: Add 50-70 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.

**11.** Repeat the elution step once, with second volume of 50-70 µl of DEPC-treated water.

## 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 Lysis Buffer and repeat the purification with new sample.<br>• Avoid warming the QVL/Carrier RNA frequently. |
| | RNA remains on the plate | • Repeat elution.<br>• Pre-heat DEPC-water to 70° C prior to elution.<br>• Incubate for 5 min with water prior to elution |
| | Plate is overloaded | • Reduce quantity of starting material. |
| Clogged well | Incomplete lysis | • Mix thoroughly after addition of QVL Lysis Buffer..<br>• Reduce amount of starting material |
| Degraded RNA | Source | • Do not freeze and thaw sample more than once.<br>• Follow protocol closely, and work quickly.<br>• Low concentration of virus in the sample |
| | RNase contamination | • Ensure not to introduce RNase during the procedure.<br>• 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.<br>• 1 X Wash Buffer II must be stored at room temperature.<br>• Repeat wash with Wash Buffer II. |
| | Inhibitors of PCR | • Use less starting material<br>• Prolong incubation with Buffer QVL to completely lyse cells |
| DNA contamination | | • Digest with RNase-free DNase and inactivate at 75°C for 5 min. |