OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E-Z 96 Viral RNA Kit

R1074-00	1 x 96 preps
R1074-01	4 x 96 preps

October 2013

For research use only. Not intended for diagnostic testing.

E-Z 96 Viral RNA Kit

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Introduction and Overview

E-Z 96 Viral RNA Kit is designed for isolation of viral RNA from acellular 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 validated with hepatitis A and 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 the reversible binding properties of the silica-based HiBind® matrix combined with the speed of mini-column spin technology or vacuum manifold, to process multiple samples quickly and efficiently. The sample is lysed under denaturing conditions that inactivate RNases and protects the intact viral RNA from degradation. After adjusting the binding conditions, the samples are transferred to the E-Z 96 RNA Plate. With a brief centrifugation or vacuum step, the samples pass through the plate and the viral RNA binds to the Hibind® matrix. After two wash steps, purified viral RNA is 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 if they are present in the sample. Acellular body fluids are recommended.

Sample Volumes: HiBind® RNA matrix can bind any RNA greater than 200 nt. Yield will depend on the sample source and condition. The protocol is optimized for use with a sample volume of 150 µL. Smaller samples should be adjusted to 150 µL with PBS or DEPC-treated 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.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- OB Protease is now supplied in a liquid form eliminating the resuspension step prior to use. OB Protease Solution can be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Product	R1074-00	R1074-01
Purifications	1 x 96	4 x 96
E-Z 96 RNA Plates	1	4
96-well Racked Microtubes (1.2 mL)	1	4
Caps for Racked Microtubes	13 x 8	52 x 8
96-well Square-well Plates (2.2 mL)*	1	2
QVL Lysis Buffer	60 mL	200 mL
RWF Wash Buffer	125 mL	300 mL
RNA Wash Buffer II	50 mL	2 x 50 mL
OB Protease Solution	1.1 mL	4.4 mL
Carrier RNA	1 mg	2 mg
AeraSeal Film	6	24
DEPC Water	20 mL	40 mL
User Manual	✓	✓

* The 96-well Square-well Plates supplied with this kit are reusable. Please refer to Page 4 for cleaning instructions.

Storage and Stability

All of the E-Z 96 Viral RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. QVL Lysis Buffer/carrier RNA solution is stable for up to 6 months when stored at 2-8°C. QVL/carrier RNA solution is only stable for a maximum of 14 days when stored at room temperature. It is recommended that aliquots of this buffer be made according to average usage per week. OB Protease Solution can be stored at room temperature for up to 12 months. For long-term storage, store OB Protease Solution at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in the QVL Lysis Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. DEPC Water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Cleaning of 96-well Square-well Plates

The 96-well Square-well Plates supplied with this kit are reusable. To avoid cross-contamination, rinse the plates thoroughly with tap water after each use. Soak the plates in 0.5M HCl for 5 minutes then wash thoroughly with distilled water. The 96-well Square-well Plates also can be autoclaved following washing.

Isolation of Cellular, Bacterial, or Viral DNA from Urine

The QVL Lysis Buffer can inactivate numerous PCR inhibitors found in urine. 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 a very low number of cells, bacteria, and viruses, samples often need to be concentrated to a final volume of 150 µL.

- Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R1074-00	200 mL
R1074-01	200 mL

- Resuspension of carrier RNA with QVL Lysis Buffer.
  1. Add 1 mL QVL Lysis Buffer to the vial of carrier RNA.
  2. Shake the vial to completely dissolve the carrier RNA.
  3. Transfer the dissolved carrier RNA to the bottle of QVL Lysis Buffer.
  4. Store at 2-8°C.

Vacuum Manifold Information

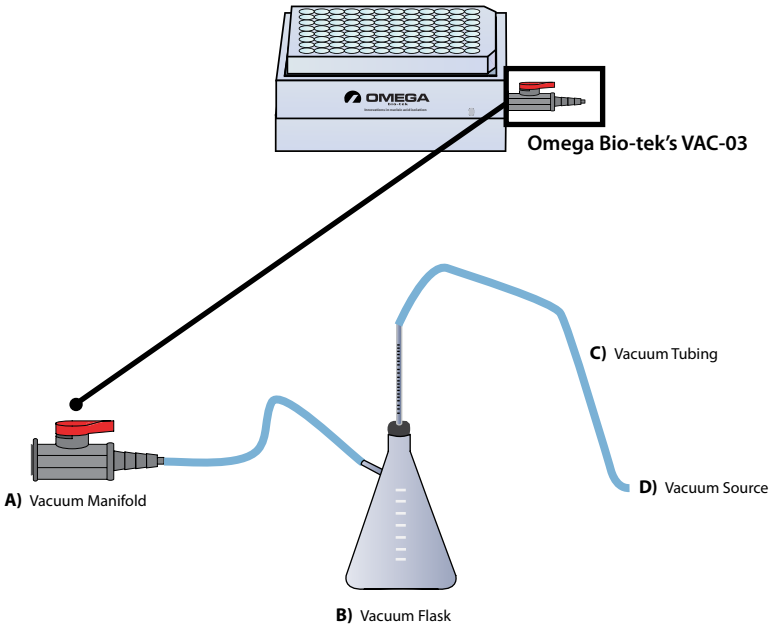
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-03	-200 to -400

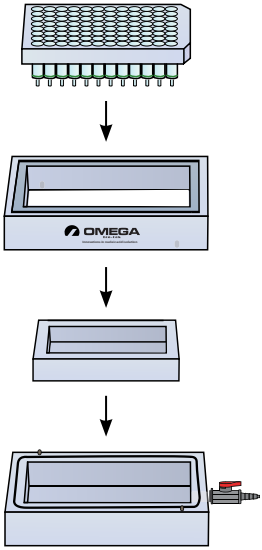
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup

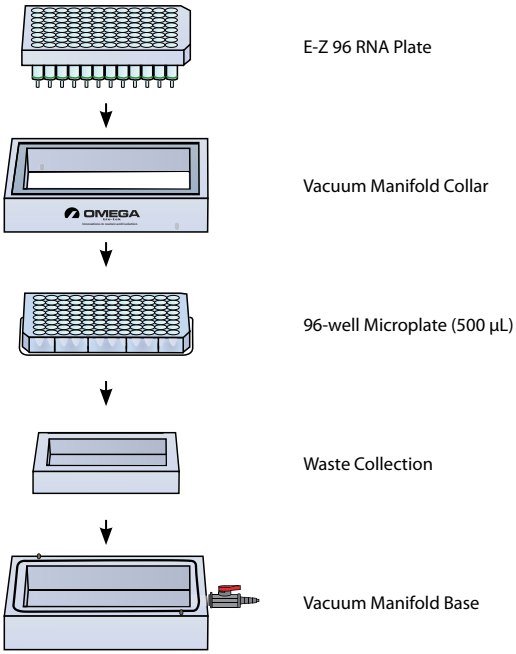


Vacuum Manifold Information

RNA Bind & Wash Setup



Standard Elution Setup



E-Z 96 Viral RNA Kit Protocol - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable 4,000 x *g* with adaptor for 96-well plates
- Vortexer
- Multichannel pipet
- RNase-free filter tips
- Reagent reservoirs for multichannel pipet
- RNase-free 1.2 mL 96-well plates
- 2 mL 96-well plates
- 100% ethanol

Before Starting:

- Prepare RNA Wash Buffer II and QVL Lysis Buffer/carrier RNA solution according to the Preparing Reagents section on Page 5.
- Equilibrate QVL Lysis Buffer/carrier RNA to room temperature before use.

1. Add 150 µL plasma, acellular body fluid, cell culture, or urine to each well of a 1.2 mL 96-well plate (not provided).

2. Add 10 µL OB Protease Solution to each well.

3. Add 500 µL QVL Lysis Buffer/carrier RNA solution to each well. Seal the plate with AeraSeal Film.

4. Keeping the 96-well plate 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. Let sit at room temperature for 10 minutes.

6. Briefly centrifuge at 500 x *g* to collect any liquid droplets from the film.

7. Remove and discard the AeraSeal Film.

8. Add 350 µL 100% ethanol to each well. Seal the plate with AeraSeal Film.

9. Vortex the plate for 30 seconds. Briefly centrifuge at 500 x *g* to collect any liquid droplets from the film. Remove and discard the AeraSeal Film.

10. Place an E-Z 96 RNA Plate onto a 96-well Square-well Plate (2.2 mL) (provided).

11. Transfer 500 µL sample from Step 8 (including any precipitate that may have formed) to each well of the E-Z 96 RNA Plate.

12. Seal the E-Z 96 RNA Plate with AeraSeal Film.

13. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

14. Discard the filtrate from the 96-well Square-well Plate.

15. Remove and reuse the AeraSeal Film in the following step.

16. Repeat Steps 11-15 until all the sample has been transferred to the E-Z 96 RNA Plate.

17. Remove and discard the AeraSeal Film.

18. Add 750 µL RWF Wash Buffer to each well. Seal the plate with AeraSeal Film.

19. Centrifuge at 4,000 x *g* for 5 minutes at room temperature. Remove and discard the AeraSeal Film.

20. Transfer the E-Z 96 RNA Plate to a clean 2 mL 96-well plate (not provided).

21. Add 500 µL RNA Wash Buffer II to each well. Seal the plate with AeraSeal Film.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

22. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

23. Discard the filtrate from the 96-well plate.

24. Remove and reuse the AeraSeal Film in the following step.

25. Repeat Steps 23-26 for a second RNA Wash Buffer II wash step.

26. Centrifuge at 4,000 x *g* for 10 minutes at room temperature.

    Note: It is important to dry the E-Z 96 RNA Plate matrix before elution. Residual ethanol may interfere with downstream applications.

27. Remove and discard the AeraSeal Film.

28. Transfer the E-Z 96 RNA Plate to the 96-well Racked Microtubes (provided).

29. Add 50-70 µL DEPC Water to each well. Seal the plate the new AeraSeal Film. Make sure to add water directly onto RNA matrix.

30. Let sit for 1 minute at room temperature.

31. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

32. Remove and reuse the AeraSeal Film in the following step.

33. Repeat Steps 30-32 for a second elution step.

34. Seal the 96-well Racked Microtubes with Caps for Racked Microtubes.

35. Store RNA at -70°C.

E-Z 96 Viral RNA Kit Protocol - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold (Cat# VAC-03)
- Vacuum source
- Multichannel pipet
- RNase-free filter tips
- Reagent reservoirs for multichannel pipet
- RNase-free 1.2 mL 96-well plates
- Sealing film
- 100% ethanol

Before Starting:

- Prepare RNA Wash Buffer II and QVL Lysis Buffer/carrier RNA according to the Preparing Reagents section on Page 5.
- Equilibrate QVL Lysis Buffer/carrier RNA to room temperature before use.

1. Add 150 µL plasma, acellular body fluid, cell culture, or urine to each well of a 1.2 mL 96-well plate (not provided).

2. Add 10 µL OB Protease Solution to each well.

3. Add 500 µL QVL Lysis Buffer/carrier RNA solution to each well. Seal the plate the AeraSeal Film.

4. Keeping the 96-well plate 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. Let sit at room temperature for 10 minutes.

6. Remove and discard the AeraSeal Film.

7. Add 350 µL 100% ethanol to each well. Pipet up and down 4 times to mix thoroughly.

8. Assemble the vacuum manifold according to the manufacturer's instructions.

9. Transfer 500 µL sample from Step 6 (including any precipitate that may have formed) to each well of the E-Z 96 RNA Plate.

   Note: Seal any unused wells with sealing film (not AeraSeal Film).

10. Turn on the vacuum source to draw the sample through the plate.

11. Turn off the vacuum.

12. Repeat Steps 8-10 until all the sample has been transferred to the E-Z 96 RNA Plate.

13. Add 750 µL RWF Wash Buffer to each well.

14. Turn on the vacuum source to draw the RWF Wash Buffer through the plate.

15. Turn off the vacuum.

16. Lift the Vacuum Manifold Collar containing the E-Z 96 RNA Plate from the Vacuum Manifold Base and empty the waste. Reassemble the manifold.

17. Add 500 µL RNA Wash Buffer II to each well.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

18. Turn on the vacuum source to draw the RNA Wash Buffer II through the plate.

19. Turn off the vacuum.

20. Repeat Steps 16-18 for a second RNA Wash Buffer II wash step.

21. Place the E-Z 96 RNA Plate on a stack of paper towels and tap the bottom (tip side) several times to remove any residual ethanol.

22. Return the E-Z 96 RNA Plate to the vacuum manifold.

23. Turn on the vacuum source for 15 minutes.

24. Turn off the vacuum.

25. Replace the Waste Tray with the 96-well Racked Microtubes (provided) and reassemble the manifold.

26. Add 50-70 µL DEPC Water to each well. Seal the E-Z 96 RNA Plate with new AeraSeal Film. Make sure to add the DEPC Water directly to the center of each well.

27. Let sit for 1 minute at room temperature.

28. Turn on the vacuum source for 5 minutes.

29. Turn off the vacuum.

30. Repeat Steps 25-28 for a second elution step.

31. Seal the 96-well Racked Microtubes with Caps for Racked Microtubes.

32. Store RNA at -70°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
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 a new sample. • Avoid warming the QVL/carrier RNA. • Repeat elution. • Preheat DEPC Water to 70°C prior to elution. • Let sit for 5 minutes with DEPC Water prior to elution
	Plate is overloaded	Reduce the amount of starting material.
Problem	Cause	Solution
Clogged well	Incomplete lysis	• Mix thoroughly after addition of QVL Lysis Buffer. • Reduce the amount of the starting material.
Problem	Cause	Solution
Degraded RNA	Source	• Do not freeze/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	Cause	Solution
Problem in downstream applications	Salt carryover during elution	• Ensure RNA Wash Buffer II has been diluted with 100% ethanol as instructed. • RNA Wash Buffer II must be stored at room temperature. • Repeat wash with RNA Wash Buffer II.
	Inhibitors of PCR	• Use less starting material. • Increase incubation with QVL Lysis Buffer to completely lyse cells.
Problem	Cause	Solution
DNA contamination		Digest with RNase-free DNase and inactivate at 75°C for 5 minutes.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
E-Z 96 Vacuum Manifold	VAC-03
RNA Wash Buffer II (25 mL)	PR031
DEPC Water (100 mL)	PR032
RNase-free DNase Set (50 preps)	E1091
RNase-free DNase Set (200 preps)	E1091-02
96-well Square-well Plate (2.2 mL)	EZ9602
Sealing Film	AC1200
AeraSeal Film	AC1201

Notes: