Ver: 2211

96-Well Virus RNA Isolation Kit

（BW-R6816）

Contents

Kit Contents

Catalog#	BW-R6816-00	BW-R6816-01	BW-R6816-02
Preps	1	4	20
Buffer LY	60 mL	240 mL	1200 mL
RNA carrier	68 µL	272 µL	680 µL
Buffer RB	50 mL	200 mL	1000 mL
RNA Wash Buffer	30 mL	3×40 mL	6×100 mL
DEPC-Treated ddH2O	6mL	24 mL	120mL
96-well RNA Plate	1	4	20
1.6 mL 96-well plate	1	4	20
2.2 mL 96-well plate	1	4	20
96-Well Collection Plate	1	4	20
AeraSeal Film	4	16	80
User Manual	1	1	1

* Add 120 mL (BW-R6816-00) or 160 mL (BW-R6816-01) or 400 mL (BW-R6816-02) 96-100% ethanol to each RNA Wash Buffer bottle before use.

Introduction

The 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 96 Viral RNA method is ready for applications such as RT-PCR*.

The 96 Viral RNA Kit uses the reversible binding properties of the silica-based 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 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.

Storage and Stability

All components can be stored at 4-28℃. All kit components are guaranteed for 12 months from the date of production.

Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

Important Notes

✪Determine the volume of Buffer LY to be used and add 20 µL of β - mercaptoethanol (β -ME) per 1 mL Buffer LY before use. Buffer LY contains β -ME can be stored at room temperature for up to 1 month.

✪Crystals may form in Buffer LY, dissolve the precipitates at 37 oC before use.

✪Add 120 mL (BW-R6816-00),160mL (BW-R6816-01), ,400mL (BW-R6816-02) 100% ethanol to each RNA Wash Buffer before use. The ethanol is 80%.

✪Add 612 µL (BW-R6816-00), 2448 µL (BW-R6816-01), 6120 µL (BW-R6816-02) DEPC Water to the vial of RNA carrier. Dissolve RNA carrier completely. Store aliquots at -20℃. Do not freeze-thaw the aliquots of carrier RNA more than 3 times.

✪Table 1.1

Number of Preps	Amount of Buffer LY (mL)	Amount of RNA carrier (µL)
2	1	11.2
4	2	22.4
10	5	56

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
- 100% ethanol

Protocol:

1. Prepare a master mix of Buffer LY and RNA carrier according to table 1.1. The mixture of **Buffer LY/RNA carrier** is stable at **2-8 ℃for 48 hours.**
2. Pipet 100-300 µL plasma or serum to each well of a 1.6 mL 96-well plate. and add **2 volumes Buffer LY** into the plasma or serum. Seal the plate with AeraSeal Film.

   Note: Add 20 µL of β-mercaptoethanol per 1 mL Buffer LY before use.
3. 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. Let sit at room temperature for 10 minutes.
4. Add **0.5 volume 100% ethanol** into the lysate (for example: 250 µL 100% ethanol for 500 µL lysate) to each well. pipet 5 times to mix the solution. or seal the plate with AeraSeal Film.Vortex the plate for 30 seconds.
5. Transfer sample from Step 4 to each well of the 96 RNA Plate.Seal the 96 RNA Plate with AeraSeal Film. Transfer the 96 RNA Plate to a clean 2.2 mL 96-well plate .Centrifuge at 4,000 x *g* for 5 minutes at room temperature.Discard the filtrate from the 96-well Square-well Plate.
6. Remove and reuse the AeraSeal Film in the following step. Add **500 µL Buffer RB** to to each well of the 96 RNA Plate.Seal the 96 RNA Plate with AeraSeal Film. Transfer the 96 RNA Plate to a clean 2.2 mL 96-well plate .Centrifuge at 4,000 x *g* for 5 minutes at room temperature.Discard the filtrate from the 2.2 mL 96-well plat.
7. Remove and reuse the AeraSeal Film in the following step. Add **650 µL RNA Wash Buffer** to to each well of the 96 RNA Plate.Seal the 96 RNA Plate with AeraSeal Film.Centrifuge

at 4,000 x *g* for 5 minutes at room temperature.Discard the filtrate from the 2.2 mL 96-well plat. Repeat 7.

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

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

9. Remove and discard the AeraSeal Film.Transfer the 96 RNA Plate to the 96- Well Collection Plate.Add 30-50 µL DEPC-Treated ddH2O to each well. Seal the plate with the new AeraSeal Film. Make sure to add water directly onto RNA matrix.Let sit for 1 minute at room temperature.Centrifuge at 4,000 x g for 5 minutes at room temperature. Store the RNA solution at -20℃.

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
- Sealing film
- 100% ethanol

Protocol:

1. Prepare a master mix of Buffer LY and RNA carrier according to table 1.1. The mixture of **Buffer LY/RNA carrier** is stable at **2-8 ℃for 48 hours.**
2. Pipet 100-300 µL plasma or serum to each well of a 1.6 mL 96-well plate. and add **2 volumes Buffer LY/RNA carrier** into the plasma or serum. Seal the plate with AeraSeal Film.

   Note: Add 20 µL of β-mercaptoethanol per 1 mL Buffer LY before use.
3. 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. Let sit at room temperature for 10 minutes.

4. Add **0.5 volume** **100% ethanol** into the lysate (for example: 250 µL 100% ethanol for 500 µL lysate) to each well. pipet 5 times to mix the solution. or seal the plate with AeraSeal Film.Vortex the plate for 30 seconds.
5. Transfer sample from Step 3 to each well of the 96 RNA Plate.Seal the 96 RNA Plate with Seal Film. Transfer the 96 RNA Plate to a clean 2.2 mL 96- well plate .
6. Assemble the vacuum manifold according to the manufacturer's instructions. Turn on the vacuum source to draw the sample through the plate.
7. Remove and reuse the Seal Film in the following step. Add **500 µL** **Buffer RB** to to each well of the 96 RNA Plate.Seal the 96 RNA Plate with AeraSeal Film. Transfer the 96 RNA Plate to a clean 2.2 mL 96-well plate. Turn on the vacuum source to draw the **Buffer RB** through the plate, Discard the filtrate from the 2.2 mL 96-well Plate.
8. Remove and reuse the Seal Film in the following step. Add **650 µL** **RNA Wash Buffer** to each well of the 96 RNA Plate.Seal the 96 RNA Plate with Seal Film. Turn on the vacuum source to draw the **RNA Wash Buffer** through the plate. Discard the filtrate from the 2.2 mL 96-well Plate. Repeat 8.
9. Turn on the vacuum source for 15 minutes.

   Note: It is important to dry the 96 RNA Plate matrix before elution. Residual ethanol may interfere with downstream applications.
10. Remove and discard the AeraSeal Film.Transfer the 96 RNA Plate to the 96- Well Collection Plate.Add 30-50 µL DEPC-Treated ddH2O to each well. Seal the plate with the new AeraSeal Film. Make sure to add water directly onto RNA matrix.Let sit for 1 minute at room temperature. Turn on the vacuum source for 5 minutes. Store the RNA solution at -20℃.

Trouble Shooting Guide

Problem	Possible reason	Suggested Improvement
Clogged well	Incomplete lysis	Mix thoroughly after addition of LY Buffer.
		Reduce the amount of the starting material.
Degraded RNA	Source	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 carryover during elution	Ensure RNA Wash Buffer has been diluted with 100% ethanol as instructed.
		RNA Wash Buffer must be stored at room temperature.
		Repeat wash with RNA Wash Buffer.
	Inhibitors of PCR	Use less starting material.
		Increase incubation with LY Buffer to completely lyse cells.
DNA contaminatin		Digest with RNase-free DNase and inactivate at 75°C for 5 minutes.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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