96-Well Viral DNA/RNA Extraction Kit

For research use only

Sample: 200 µl sample (plasma, serum, body fluid or the supernatant of viral infected cell cultures)

Yield: up to 30 µg/well

Format: 96-well plates

Operation: vacuum manifold/centrifuge

Operation time: within 30 minutes

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Introduction

The 96-Well Viral DNA/RNA Extraction Kit was designed specifically for high-throughput purification of viral DNA/RNA from cell-free samples such as serum, plasma, body fluids and the supernatant of viral infected cell cultures. DNA/RNA viruses are lysed quickly and efficiently when exposed to the lysis buffer which is a highly concentrated solution of chaotropic salt. When AD Buffer and ethanol are combined, the mixture creates optimal conditions for binding Nucleic Acid to the glass fiber matrix (1) of the Viral DNA/RNA Binding Plate. Contaminants such as salts, metabolites and soluble macromolecular cellular components are removed in the Wash step. The nucleic acid can be eluted in RNase-free water and is then ready for use in subsequent reactions including, Real-time PCR/RT-PCR, Automated Fluorescent DNA Sequencing, PCR, and other enzymatic reactions. The detection limit for certain viruses depends on the sensitivity of individual PCR or RT-PCR assay.

Quality Control

The quality of the 96-Well Viral DNA/RNA Extraction Kit is tested on a lot-to-lot basis by isolating viral DNA/RNA from a 200 µl plasma sample.

Kit Contents

Name	VNP02	VNP04	VNP10
VB Lysis Buffer	100 ml	200 ml	500 ml
AD Buffer[1] (Add Ethanol)	13 ml (100 ml)	26 ml (200 ml)	26 ml x 2 (200 ml x 2)
W1 Buffer	130 ml	130 ml	130 ml x 3
Wash Buffer[2] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	50 ml x 3 (200 ml x 3)
RNase-free Water	30 ml	30 ml	60 ml
Viral DNA/RNA Binding Plate	2 pcs	4 pcs	10 pcs
2 ml Collection Plate	2 pcs	4 pcs	10 pcs
Lysis Plate	2 pcs	4 pcs	10 pcs
PCR Plate	2 pcs	4 pcs	10 pcs
Adhesive film	10 pcs	20 pcs	50 pcs

Order Information

Product Name	Package size	Cat. No.
Viral Nucleic Acid Extraction Kit II (200 µl)	50/100/300 preps	VR050/100/300
Viral Nucleic Acid Extraction Kit III (1 ml)	50/100/300 preps	VI050/100/300
96-Well Viral DNA/RNA Extraction Kit	2/4/10 X 96 Wells	VNP02/04/10
Vacuum Manifold (Accessories)	1 SET	ZVF01

[1]Add absolute ethanol (see the bottle label for volume) to the AD Buffer prior to initial use
[2]Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

Caution

VB Lysis Buffer and W1 Buffer contain chaotropic salt. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* 76, 615

96-Well Viral DNA/RNA Extraction Kit Vacuum Protocol

- Add absolute ethanol (see the bottle label for volume) to AD Buffer prior to initial use
- Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
- Additional requirements: multi-well plate vacuum manifold, 2 ml collection plate

Step 1 Lysis	• Add **400 µl of VB Lysis Buffer** to each well of a **Lysis Plate**. • Transfer 200 µl of sample (plasma, serum, body fluids, the supernatant of a viral infected cell culture) to each well of the **Lysis Plate**. • Seal the plate with **Adhesive Film** then mix by vortex. • Incubate at room temperature for 10 minutes.
Step 2 Nucleic Acid Binding	• Remove the **Adhesive Film** from the **Lysis Plate**. • Add **450 µl of AD Buffer** (make sure ethanol was added) to each sample lysate. • Seal the plate with a new **Adhesive Film** then vortex immediately. • Remove the vacuum manifold receiver plate and place a **2 ml Collection Plate** on the base. • Return the receiver plate to its resting position. • Place a **Viral DNA/RNA Binding Plate** on top of the receiver plate and fit both together tightly. • Remove the **Adhesive Film** from the **Lysis Plate**. • Transfer 600 µl of lysate mixture to each well of the **Viral DNA/RNA Binding Plate**. • Turn on the vacuum pump at 800 mbar for a few seconds or until the wells empty. • Turn off the vacuum pump and transfer the remaining lysate mixture to each well. • Turn on the vacuum pump at 800 mbar for a few seconds or until the wells empty. • Turn off the vacuum then remove the receiver plate together with the **Viral DNA/RNA Binding Plate**. • Discard the flow-through waste in the **2 ml Collection Plate** then return it to its resting position. • Return the receiver plate and **Viral DNA/RNA Binding Plate** to their resting position.
Step 3 Wash	• Add **300 µl of W1 Buffer** to the **Viral DNA/RNA Binding Plate**. • Turn on the vacuum pump at 800 mbar for 2 minutes. • Turn off the vacuum pump then add **600 µl of Wash Buffer** (make sure ethanol was added) to each well of the **Viral DNA/RNA Binding Plate.** • Turn on the vacuum pump at 800 mbar for 2 minutes. • Turn off the vacuum then remove the receiver plate together with the **Viral DNA/RNA Binding Plate**. • Discard the **2 ml Collection Plate** containing the flow-through waste. • Place a clean 2 ml collection plate on the base. • Return the receiver plate and **Viral DNA/RNA Binding Plate** to their resting position. • Turn on the vacuum pump at 800 mbar for 5 minutes to remove any ethanol residue.
Step 4 Nucleic Acid Elution	• Turn off the vacuum then remove the receiver plate together with the **Viral DNA/RNA Binding Plate**. • Place a **PCR Plate** on the 2 ml collection plate. • Return the receiver plate and **Viral DNA/RNA Binding Plate** to their resting position. • Add **50 µl of RNase-free water** to the center of each well of the **Viral DNA/RNA Binding Plate**. • Let stand for at least 3 minutes to ensure the water is absorbed by the matrix. • Turn on the vacuum pump at 800 mbar for a few seconds to elute the viral DNA/RNA. • Turn off the vacuum then remove the receiver plate together with the **Viral DNA/RNA Binding Plate**. • Remove the **PCR Plate**. • Seal the **PCR Plate** with a new **Adhesive Film** and store the purified DNA/RNA at -20ºC.

96-Well Viral DNA/RNA Extraction Kit Centrifuge Protocol

- Add absolute ethanol (see the bottle label for volume) to AD Buffer prior to initial use
- Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
- Additional requirements: multi-well plate centrifugation system, 0.35 ml collection plate

Step1 Lysis	• Add **400 µl of VB Lysis Buffer** to each well of a **Lysis Plate**. • Transfer 200 µl of sample (plasma, serum, body fluids, the supernatant of a viral infected cell culture) to each well of the **Lysis Plate**. • Seal the plate with **Adhesive Film** and mix by vortex. • Incubate at room temperature for 10 minutes.
Step 2 Nucleic Acid Binding	• Remove the **Adhesive Film** from the **Lysis Plate**. • Add **450 µl of AD Buffer** (make sure ethanol was added) to each sample lysate. • Seal the plate with a new **Adhesive Film** then vortex immediately. • Place a **Viral DNA/RNA Binding Plate** on a **2 ml Collection Plate**. • Remove the **Adhesive Film from the Lysis Plate**. • Transfer 600 µl of the lysate mixture to each well of the **Viral DNA/RNA Binding Plate**. • Centrifuge for 5 minutes at 3,000 x g then discard the flow-through. • Place the **Viral DNA/RNA Binding Plate** back on the **2 ml Collection Plate**. • Transfer the remaining lysate mixture to each well of the **Viral DNA/RNA Binding Plate.** • Centrifuge for 5 minutes at 3,000 x g again then discard the flow-through. • Place the **Viral DNA/RNA Binding Plate** back on the **2 ml Collection Plate**.
Step 3 Wash	• Add **300 µl of W1 Buffer** to each well of the **Viral DNA/RNA Binding Plate**. • Centrifuge for 3 minutes at 3,000 x g then discard the flow-through. • Place the **Viral DNA/RNA Binding Plate** back on the **2 ml Collection Plate**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) to each well of the **Viral DNA/RNA Binding Plate.** • Centrifuge for 1 minute at 3,000 x g then discard the flow-through. • Place the **Viral DNA/RNA Binding Plate** back on the **2 ml Collection Plate**. • Centrifuge for 10 minutes at 3,000 x g to remove any ethanol residue.
Step 4 Nucleic Acid Elution	• Place the **Viral DNA/RNA Binding Plate** on a 0.35 ml collection plate. • Add **50 µl of RNase-free water** to the center of each well of the **Viral DNA/RNA Binding Plate**. • Let stand for at least 3 minutes to ensure the water is absorbed by the matrix. • Centrifuge for 1 minute at 3,000 x g. • Seal the 0.35 ml collection plate with a new **Adhesive Film** and store the purified DNA/RNA at -20ºC.