

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
Operation time	: 40 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 the AD Buffer and ethanol are combined, the mixture creates the optimum condition 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 to 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	13 ml	26 ml	26 ml x 2
(Add Ethanol)	(100 ml)	(200 ml)	(200 ml x 2)
W1 Buffer	130 ml	130 ml	130 ml x 3
Wash Buffer ²	25 ml	50 ml	50 ml x 3
(Add Ethanol)	(100 ml)	(200 ml)	(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

¹Add absolute ethanol to the AD Buffer prior to initial use (see the bottle label for volume).

²Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Caution

VB Lysis Buffer and W1 Buffer contain chaotropic salt which is a harmful irritant. 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 to the AD Buffer prior to initial use (see the bottle label for volume).
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: multi-well plate vacuum manifold, 2 ml collection plate

Step 1 Lysis	<ul style="list-style-type: none">● Add 400 µl of VB Lysis Buffer to each well of a Lysis Plate.● Transfer 200 µl of a serum sample (plasma, body fluids, the supernatant of a viral infected cell culture) to each well of the pre-filled Lysis Plate.● Seal the plate with Adhesive Film and mix by vortex.● Incubate at room temperature for 10 minutes.
Step 2 Nucleic Acid Binding	<ul style="list-style-type: none">● Remove the Adhesive Film on the Lysis Plate and add 450 µl of AD Buffer (ethanol added) to each sample lysate.● Seal the plate with a new Adhesive Film and vortex immediately.● Open the top cap of the vacuum manifold and place a 2 ml Collection Plate in the chamber and close the cap.● Place a Viral DNA/RNA Binding Plate onto the gasket of the vacuum manifold and fit both together tightly.● Remove the Adhesive Film and transfer 600 µl of the 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 and lift up the top portion of the vacuum manifold carrying the Viral DNA/RNA Binding Plate from the base. Discard the flow-through waste in the 2 ml Collection Plate.● Reassemble the plate and the vacuum manifold with the Viral DNA/RNA Binding Plate.
Step 3 Wash	<ul style="list-style-type: none">● 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 and add 600 µl of Wash Buffer (ethanol 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 pump. Lift up the top portion of the vacuum manifold carrying the Viral DNA/RNA Binding Plate and discard the 2 ml Collection Plate containing the flow-through waste.● Place a clean 2 ml Collection Plate into the chamber and reassemble the vacuum manifold with the Viral DNA/RNA Binding Plate.● Turn on the vacuum pump at 800 mbar for 5 minutes to remove any ethanol residue.
Step 4 Nucleic Acid Elution	<ul style="list-style-type: none">● Turn off the vacuum and lift up the top portion of the vacuum manifold carrying the Viral DNA/RNA Binding Plate from the base. Place a PCR Plate on the 2 ml Collection Plate and reassemble the vacuum manifold with the Viral DNA/RNA Binding Plate.● Add 50 µl of RNase-free water to the center of each well of the Viral DNA/RNA Binding Plate.● Let stand for 3 minutes or until 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 pump and lift up the top portion of the vacuum manifold and remove the PCR Plate.● Seal the plate with a new Adhesive Film and store the purified DNA/RNA at -20°C.

96-Well Viral DNA/RNA Extraction Kit Centrifuge Protocol

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

Step 1 Lysis	<ul style="list-style-type: none">● Add 400 µl of VB Lysis Buffer to each well of a Lysis Plate.● Transfer 200 µl of a serum sample (plasma, body fluids, the supernatant of a viral infected cell culture) to each well of the pre-filled Lysis Plate.● Seal the plate with Adhesive Film and mix by vortex.● Incubate at room temperature for 10 minutes.
Step 2 Nucleic Acid Binding	<ul style="list-style-type: none">● Remove the Adhesive Film on the Lysis Plate and add 450 µl of AD Buffer (ethanol added) to each sample lysate.● Seal the plate with a new Adhesive Film and vortex immediately.● Place a Viral DNA/RNA Binding Plate on a 2 ml Collection Plate.● Remove the Adhesive Film and 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.● Discard the flow-through and 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.● Discard the flow-through and place the Viral DNA/RNA Binding Plate back on the 2 ml Collection Plate.
Step 3 Wash	<ul style="list-style-type: none">● Add 300 µl of W1 Buffer to the Viral DNA/RNA Binding Plate.● Centrifuge for 3 minutes at 3,000 x g.● Discard the flow-through and place the Viral DNA/RNA Binding Plate back on the 2 ml Collection Plate.● Add 600 µl of Wash Buffer (ethanol added) to each well of the Viral DNA/RNA Binding Plate.● Centrifuge for 1 minute at 3,000 x g.● Discard the flow-through and 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	<ul style="list-style-type: none">● 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 3 minutes or until the water is absorbed by the matrix.● Centrifuge for 1 minute at 3,000 x g.● Seal the plate with a new Adhesive Film and store the purified DNA/RNA at -20°C.