

Viral Nucleic Acid Extraction Kit II



For research use only

- Sample** : 200 µl sample (plasma, serum, body fluid or the supernatant of viral infected cell cultures)
- Format** : spin column
- Rxns** : 50, 100 and 300
- Operation time** : 40 minutes



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Introduction

The Viral Nucleic Acid Extraction Kit II was designed specifically for 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 using the lysis buffer which is a highly concentrated solution of chaotropic salt. When combined with ethanol, the AD Buffer creates optimum conditions for Nucleic Acid binding to the glass fiber matrix of the column (1). Contaminants such as salts, metabolites and soluble macromolecular cellular components are removed in the Wash step. Nucleic Acid is 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. This protocol is recommended for parallel purification of viral DNA including HBV and CMV and viral RNA including HCV, HIV, and HTLV. The detection limit for certain viruses depends on the sensitivity of individual PCR or RT-PCR assays.

Quality Control

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

Kit Contents

Name	VR004	VR050	VR100	VR300
VB Lysis Buffer	2 ml	30 ml	60 ml	130 ml
AD Buffer ¹	0.5 ml	4 ml	8 ml	24 ml
(Add Ethanol)	(4 ml)	(30 ml)	(60 ml)	(180 ml)
W1 Buffer	2 ml	30 ml	50 ml	130 ml
Wash Buffer ²	1 ml	12.5 ml	25 ml	50 ml
(Add Ethanol)	(4 ml)	(50 ml)	(100 ml)	(200 ml)
RNase-free Water	1 ml	6 ml	6 ml	30 ml
VB Column	4 pcs	50 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	100 pcs	200 pcs	600 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 contains 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

Viral Nucleic Acid Extraction Kit II 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: absolute ethanol, microcentrifuge tubes (DNase and RNase free), PBS (phosphate-buffered saline)

Step1 Lysis	<ul style="list-style-type: none">● Transfer a 200 µl sample (serum, plasma, body fluids or the supernatant of a viral infected cell culture) into a 1.5 ml microcentrifuge tube. If the prepared sample is less than 200 µl, adjust the sample volume to 200 µl with PBS buffer.● Add 400 µl of VB Lysis Buffer to the sample and mix by vortex.● Incubate at room temperature for 10 minutes.
Step 2 Nucleic Acid Binding	<ul style="list-style-type: none">● Add 450 µl of AD Buffer (ethanol added) to the sample lysate and shake vigorously.● Place a VB Column in a 2 ml Collection Tube.● Transfer 600 µl of the lysate mixture to the VB Column.● Centrifuge at 14-16,000 x g for 1 minute.● Discard the flow-through and place the VB Column back in the 2 ml Collection Tube.● Transfer the remaining lysate mixture to the VB Column.● Centrifuge at 14-16,000 x g for 1 minute.● Discard the 2 ml Collection Tube containing the flow-through and transfer the VB Column to a new 2 ml Collection Tube.
Step 3 Wash	<ul style="list-style-type: none">● Add 400 µl of W1 Buffer to the VB Column.● Centrifuge at 14-16,000 x g for 30 seconds.● Discard the flow-through and place the VB Column back in the 2 ml Collection Tube.● Add 600 µl of Wash Buffer (ethanol added) to the VB Column.● Centrifuge at 14-16,000 x g for 30 seconds.● Discard the flow-through and place the VB Column back in the 2 ml Collection Tube.● Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 4 Nucleic Acid Elution	<ul style="list-style-type: none">● Place the dried VB Column in a clean 1.5 ml microcentrifuge tube.● Add 50 µl of RNase-free water to the center of the VB Column matrix.● Let stand for 3 minutes or until the water is absorbed by the matrix.● Centrifuge at 14-16,000 x g for 1 minute to elute the purified nucleic acid.