

# Viral Nucleic Acid Extraction Kit III



*For research use only*

*Purification of Clinical Viral DNA/RNA with Short Internal Control (IC)*

- Sample Size** : 1 ml of serum or plasma  
**Format** : spin column  
**Rxns** : 50, 100, 300  
**Operation time** : 55 minutes



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## Introduction

The Viral Nucleic Acid Extraction Kit III was designed specifically for simultaneous purification of viral DNA/RNA from serum or plasma samples. The method is comprised of concentrating viral particles from serum, lysis of viral particles, viral DNA/RNA binding to the surface of the glass fiber membrane (1), and releasing of DNA/RNA into the release buffer.  $10^1$ - $10^9$  copies of viral DNA/RNA can be purified from 1 ml of serum within 1 hour. The purified viral DNA/RNA can be used directly in qPCR and qRT-PCR assays.

## Quality Control

The quality of Viral Nucleic Acid Extraction Kit III is tested on a lot-to-lot basis by isolating viral DNA/RNA from a 1 ml serum sample.

### Kit Contents

Name	VI004	VI050	VI100	VI300
PP Buffer	1 ml	12 ml	25 ml	70 ml
LS Buffer <sup>1</sup>	1 ml	6 ml	12 ml	40 ml
Wash Buffer <sup>2</sup> (Add Ethanol)	1 ml (4 ml)	5 ml (20 ml)	12.5 ml (50 ml)	25 ml (100 ml)
Acid Buffer	1 ml	1 ml	1 ml	2 ml
Release Water	1.5 ml	3 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

<sup>1</sup>If precipitates have formed in the LS Buffer, warm the buffer in a 37°C water bath to dissolve.

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

## Caution

Buffers contain harmful irritants. 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 III Protocol

- If precipitates have formed in the LS Buffer, warm the buffer in a 37°C water bath to dissolve.
- Additional requirements: microcentrifuge tubes, absolute ethanol, 3 M NaOAc pH 5.2, Isopropanol, internal control (IC)
- Prior to using this kit, preheat the Release Water to 65°C.

Step 1 Sample Preparation	<ul style="list-style-type: none"> <li>● Concentrate the virus by adding <b>150 µl of PP Buffer</b> to 1 ml of serum or plasma, and mix well (if the sample volume is less than 1 ml, 150 µl of PP Buffer is still required).</li> <li>● Let stand at room temperature for 30 minutes.</li> <li>● Centrifuge at 12,000 rpm for 15 minutes.</li> <li>● Remove the supernatant and save the viral ppt (to purify genomic DNA by HIV and HTLV Proviral DNA Integration from whole blood samples, 200-500 µl of whole blood is first lysed by 3 x RBC Lysis Buffer, centrifuged at 3,000 rpm for 15 minutes, followed by cell ppt processing).</li> </ul>
Step 2 Lysis	<ul style="list-style-type: none"> <li>● Mix <b>100 µl of LS Buffer</b> with 1 µl of IC (short ds DNA, E3/µl) and vortex.</li> <li>● Add 100 µl of the mixture into the viral ppt, vortex and incubate at room temperature for 5 minutes.</li> </ul>
Step 3 Nucleic Acid Binding	<ul style="list-style-type: none"> <li>● Add 234 µl of absolute ethanol to the mixture from step 2 and mix by shaking 10 times.</li> <li>● Place a <b>VB Column</b> in a <b>2 ml Collection Tube</b> and transfer the mixture to the <b>VB column</b>.</li> <li>● Centrifuge at 12,000 rpm for 30 seconds.</li> <li>● Discard the <b>2 ml Collection Tube</b> containing the flow-through and transfer the <b>VB Column</b> to a new <b>2 ml Collection Tube</b>.</li> </ul>
Step 4 Wash	<ul style="list-style-type: none"> <li>● Add <b>200 µl of Wash Buffer</b> to the <b>VB Column</b>.</li> <li>● Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.</li> <li>● Add <b>200 µl of Wash Buffer</b> to the <b>VB Column</b> again.</li> <li>● Centrifuge again at 12,000 rpm for 30 seconds. Discard the flow-through.</li> <li>● Centrifuge at 12,000 rpm for 2 minutes to completely remove the ethanol residue.</li> </ul>
Step 5 Elution	<ul style="list-style-type: none"> <li>● Add <b>50 µl of Release Buffer</b> (preheated to 65°C) to the center of the column matrix to release the viral DNA/RNA and IC.</li> <li>● Let stand at 65°C for 3 minutes.</li> <li>● Centrifuge at 12,000 rpm for 1 minute to elute the purified viral DNA/RNA.</li> </ul>
Optional Nucleic Acid Concentration Step	<ul style="list-style-type: none"> <li>● Add <b>5 µl of Acid Buffer</b> and 50 µl of Isopropanol to the eluted product, mix well and let stand at room temperature for 10 minutes.</li> <li>● Centrifuge at 12,000 rpm for 15 minutes and carefully discard the supernatant.</li> <li>● Dissolve ppt in 5 µl of nuclease-free ddH<sub>2</sub>O.</li> <li>● Use 1 µl for PCR or qPCR using HBV/HCV/HIV/HTLV Real-time SYBR Green Screening and Quantitative PCR Kit (developed by Geneaid and BioRad, using BioRad IQ5 multicolor real-time detection system, for Real-time Screening Detection and Quantitation of Human HBV, HCV, HIV, and HTLV viruses).</li> </ul>