Viral Nucleic Acid Extraction Kit II

For research use only

Sample: up to 200 µl plasma, serum, body fluid or the supernatant of viral infected cell cultures, nasopharyngeal and oropharyngeal swabs

Format: spin column

Operation Time: within 20 minutes

Elution Volume: 50 µl

Storage: dry at room temperature (15-25ºC)

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Viral Nucleic Acid Extraction Kit II was designed specifically for efficient purification of viral DNA and viral RNA from cell-free samples such as serum, plasma, body fluids and the supernatant of viral infected cell cultures. The efficient glass fiber spin column system is optimized for nucleic acid purification from a wide variety of both DNA and RNA viruses such as HBV, CMV, HCV, HIV, and HTLV. 10^1-10^9 copies of viral DNA/RNA can be purified from 200 µl of serum within 20 minutes. 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 II is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system by isolating viral DNA/RNA from a 200 µl serum sample.

Kit Contents

Component	VR004	VR050	VR100	VR300
VB Lysis Buffer	2 ml	30 ml	60 ml	130 ml
AD Buffer[1] (Add Ethanol)	0.5 ml (4 ml)	4 ml (30 ml)	8 ml (60 ml)	24 ml (180 ml)
W1 Buffer	2 ml	30 ml	50 ml	130 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)	50 ml (200 ml)
RNase-free Water	1 ml	6 ml	6 ml	30 ml
VB Columns	4	50	100	300
2 ml Collection Tubes	8	100	200	600

[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

Order Information

Virus DNA/RNA Purification		
Product	Package Size	Cat. Number
Plant Virus RNA Kit	50/100 preps	PVR050/100
Viral Nucleic Acid Extraction Kit II	50/100/300 preps	VR050/100/300
Viral Nucleic Acid Extraction Kit III	50/100/300 preps	VI050/100/300

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Note

The Viral Nucleic Acid Extraction Kit II is optimized to eliminate the need for Carrier RNA and Internal Control (IC).

Steps to prevent RNase contamination

Disposable and nondisposable plasticware and automatic pipettes should be sterile and used only for RNA procedures.

Viral Nucleic Acid Extraction Kit Functional Test Data

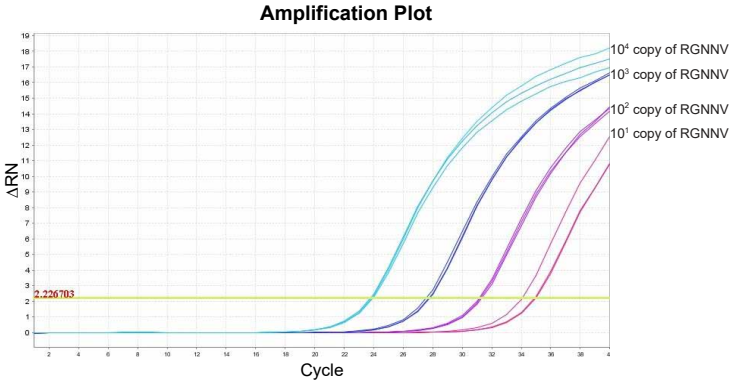


Figure 1. Virus RNA was purified from 10E1-10E4 copy number of Red Spotted Grouper Nervous Necrosis Virus (RGNNV) using the Viral Nucleic Acid Extraction Kit II (3 replications of each copy number). The purified RNA was eluted with 30 µl RNase-free Water. cDNA synthesis was carried out with a 10 µl aliquot of purified RNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche) in a final volume of 20 µl. A Real-time PCR assay was then performed with 3 µl of synthesized cDNA as template, primers (designed to amplify the T4 region on the RNA2 segment), and Fast SYBR Green PCR Master Mix using the StepOnePlus™ Real-Time PCR system (Applied Biosystems). The results confirmed that virus RNA can be successfully extracted and detected from as low as 10E1 copy number of RGNNV. The average cycle threshold (Ct): 10E4 = 23.88, 10E3 = 27.72, 10E2 = 31.22, 10E1 = 34.62. The low Ct value indicates a high number of target nucleic acid in the sample.

Viral Nucleic Acid Extraction Kit Functional Test Data

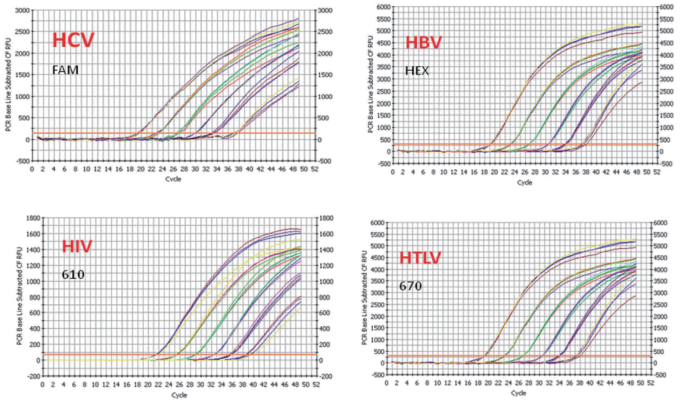


Figure 2. HBV (DNA), HCV (RNA), HIV (RNA), and HTLV (RNA) were purified from 200 µl of positive clinical serum samples using the Viral Nucleic Acid Extraction Kit II. Real-time qPCR and 1-step qRT-PCR reactions were then conducted using the ABI 7300 Sequence Detection System (3 replications of each copy number). Serum samples containing various amounts of DNA/RNA viruses ranging from 10E1 to 10E6 copies/ml were successfully detected and identified. The low Ct values indicate a high number of target nucleic acid in the sample.

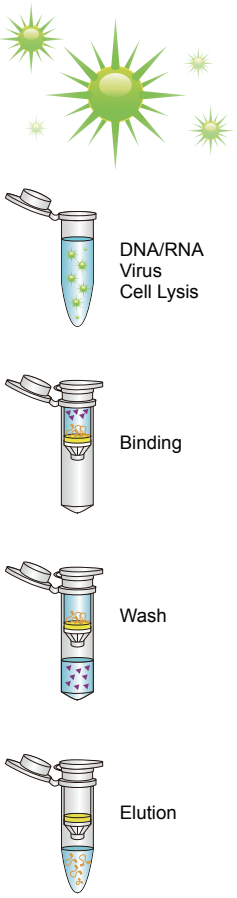
Viral Nucleic Acid Extraction Kit II Protocol

IMPORTANT BEFORE USE!

- Add absolute ethanol (see the bottle label for volume) to the AD Buffer prior to initial use
- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: absolute ethanol, microcentrifuge tubes (DNase and RNase-free), Phosphate-Buffered Saline

Step 1 Lysis	**For cell-free samples (serum, plasma, body fluids)** • Transfer **200 µl sample** to a 1.5 ml microcentrifuge tube. NOTE: If the sample is less than 200 µl, adjust the sample volume to 200 µl with PBS. • Add **400 µl of VB Lysis Buffer** to the sample then mix by vortex. • Incubate at room temperature for 10 minutes. **For nasopharyngeal and oropharyngeal swabs preserving in the transport medium** • Vortex the preservation tubes containing swabs for 1 minute. • Transfer **200 µl of medium** such as VTM, UTM and PBS to a 1.5 ml microcentrifuge tube. • Add **400 µl of VB Lysis Buffer** to the sample then mix by vortex. • Incubate at room temperature for 10 minutes.
Step 2 Nucleic Acid Binding	• Add **450 µl of AD Buffer (make sure ethanol was added)** to the sample lysate. • Shake the tube vigorously to mix. • 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 then place the **VB Column** back in the **2 ml Collection Tube.** • Transfer the **remaining** 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. • Transfer the **VB Column** to a new **2 ml Collection Tube**.
Step 3 Wash	• Add **400 µl of W1 Buffer** to the **VB Column** then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **VB Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer (make sure ethanol was 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	• 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 at least 3 minutes to ensure the **RNase-free Water** is absorbed by the matrix. • Centrifuge at 14-16,000 x g for 1 minute to elute the purified nucleic acid.

Quick Protocol



Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	• Centrifugation temperature was too low (should be 20ºC to 25ºC)
Low Yield	• DNA/RNA still bound to the VB Column membrane • Ethanol carryover
RNA Degradation	• Harvested sample not immediately stabilized • Inappropriate handling of starting material • RNase contamination