Viral Nucleic Acid Extraction Kit III

For research use only

Sample Size: up to 1 ml plasma, serum, body fluid or the supernatant of viral infected cell cultures

Format: spin column

Elution volume: 50 µl

Operation time: within 60 minutes

Storage: dry at room temperature (15-25ºC) for up to 9 months

CERTIFICATE NO. QAIC/TW/50077

ISO 9001:2008 QMS

Introduction

The Viral Nucleic Acid Extraction Kit III 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 1 ml of serum within 60 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 III is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system by isolating viral DNA/RNA from a 1 ml serum sample.

Kit Contents

Name	VI004	VI050	VI100	VI300
PT Buffer	1 ml	12 ml	25 ml	70 ml
LS Buffer[1]	1 ml	6 ml	12 ml	40 ml
Wash Buffer[2] (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	50/100/300 preps	VR050/100/300
Viral Nucleic Acid Extraction Kit III	50/100/300 preps	VI050/100/300
96-Well Viral DNA/RNA Extraction Kit	4/10 X 96 Wells	VNP04/10
Vacuum Manifold	1 SET	ZVF01

[1]If precipitates have formed in the LS Buffer, warm the buffer in a 37ºC water bath to dissolve

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

Caution

Buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Note

The Viral Nucleic Acid Extraction Kit III buffer system is optimized to eliminate the need for Carrier RNA.

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 III Protocol

- If precipitates have formed in the LS Buffer, warm the buffer in a 37ºC water bath to dissolve
- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute ethanol, isopropanol, (optional) Internal Control (IC)

Sample Preparation	• Add **150 µl of PT Buffer to 1 ml of serum or plasma** then mix well. NOTE: If the sample volume is less than 1 ml, 150 µl of PT Buffer is still required. • Let stand at room temperature for 30 minutes. • Centrifuge at 14-16,000 x g for 15 minutes. At this time, pre-heat the required **Release Water** (50 µl/sample) to 65ºC (for Step 4 Elution). • Remove the supernatant and save the viral ppt. **To purify genomic DNA by HIV and HTLV Proviral DNA Integration from whole blood samples** • Add **3 X RBC lysis buffer to 200-500 µl of whole blood**. • Centrifuge at 3,000 x g for 15 minutes followed by cell ppt processing.
Step 1 Lysis	• Add **100 µl of LS Buffer to the viral ppt** then vortex. Optional: Add 1 µl of Internal Control (short dsDNA, E3/µl) to the viral ppt then vortex. • Incubate at room temperature for 5 minutes.
Step 2 Nucleic Acid Binding	• Add **234 µl of absolute ethanol to the mixture** from step 1 then mix by shaking 10 times. • Place a **VB Column** in a **2 ml Collection Tube** then transfer the mixture to the **VB column**. • Centrifuge at 14-16,000 x g for 30 seconds. • 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 **200 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. • Add **200 µl of Wash Buffer** (make sure ethanol was added) to the **VB Column** again. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through. • Centrifuge at 14-16,000 x g for 2 minutes to completely remove the ethanol residue.
Step 4 Elution	• Add **50 µl of Release Buffer** (pre-heated to 65ºC) to the **CENTER** of the column matrix to release the viral DNA/RNA. • Let stand at 65ºC for 3 minutes. • Centrifuge at 14-16,000 x g for 1 minute to elute the purified viral DNA/RNA.
Optional Nucleic Acid Concentration Step	• Add **5 µl of Acid Buffer and 50 µl of isopropanol** to the eluted product and mix well. • Let stand at room temperature for 10 minutes. • Centrifuge at 14-16,000 x g for 15 minutes then carefully discard the supernatant. • Dissolve ppt in **5 µl of nuclease-free ddH_2O**. • Use **1 µl for PCR or qPCR**.

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