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IBI SCIENTIFIC

INSTRUCTION MANUAL

Viral Nucleic Acid Extraction Kit II

IB47400 (4 prep sample kit)
IB47401 (50 prep kit)
IB47402 (100 prep kit)
IB47403 (300 prep kit)

Advantages

Sample: up to 200 µl plasma, serum, body fluid or the supernatant of viral infected cell cultures
Format: spin column
Operation Time: within 20 minutes
Elution Volume: 50 µl
Storage: dry at room temperature (15-25°C)

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	IB47400	IB47401	IB47402	IB47303
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

Product	Package Size	Catalogue #
Total RNA Mini Kit (Blood/Cell)	50/100/300 preps	IB47321/322/323
Total RNA Mini Kit (Tissue)	50/100/300 preps	IB47301/302/303
Total RNA Mini Kit (Plant)	50/100/300 preps	IB47341/342/343
rBAC Mini RNA Bacteria Kit	100/300 preps	IB47421/422
rYeast Total RNA Mini Kit	100/300 preps	IB47411/412
miRNA Isolation Kit	100 preps	IB47371
IBI Isolate	100/200 rxns	IB47601/302
IBI Tri-Isolate	100/200 rxns	IB47631/632
RNA Pure Kit	50/100 rxns	IB47641/642

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

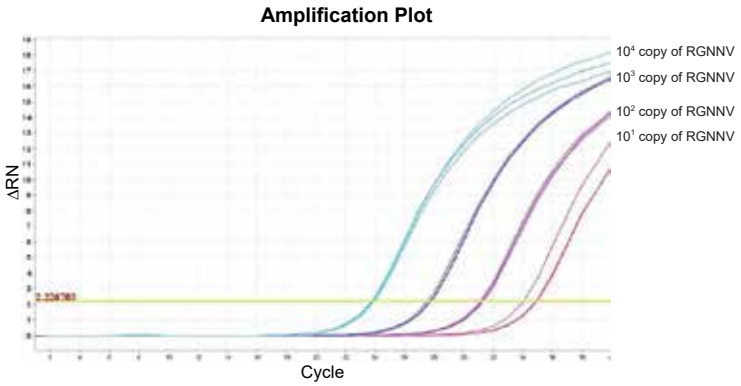
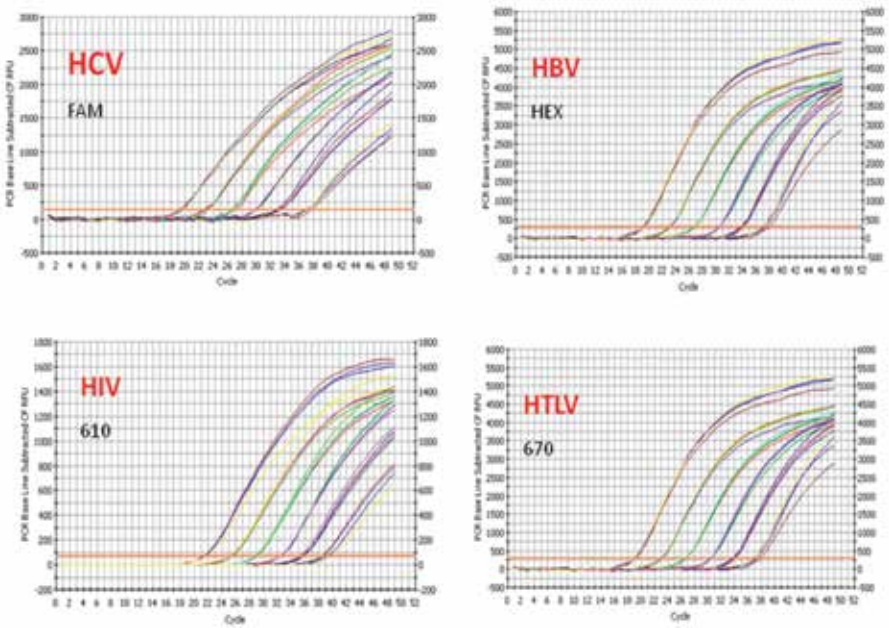


Figure 1. Virus RNA was purified from 10E1-10E4 copy number of Red Spotted Grouper Nervous Necrosis Virus (RGNNV) using the Viral Nucleic Acid Extraction Kit II (3 replications of each copy number). The purified RNA was eluted with 30 µl RNase-free Water. cDNA synthesis was carried out with a 10 µl aliquot 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.



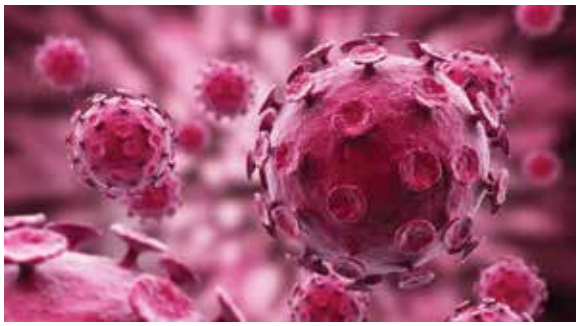


Figure 2. HBV (DNA), HCV (RNA), HIV (RNA), and HTLV (RNA) were purified from 200 µl of positive clinical serum samples using the Viral Nucleic Acid Extraction Kit II. Real-time qPCR and 1-step qRT-PCR reactions were then conducted using the ABI 7300 Sequence Detection System (3 replications of each copy 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.

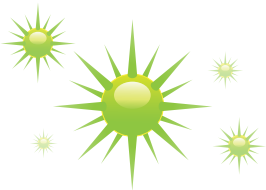
Viral Nucleic Acid Extraction Kit II Protocol

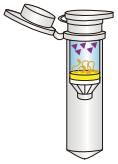
IMPORTANT BEFORE USE!

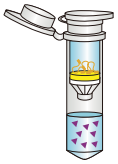
- Add absolute ethanol (see the bottle label for volume) to the AD Buffer prior to initial use
- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: absolute ethanol, microcentrifuge tubes (DNase and RNase-free), Phosphate-Buffered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

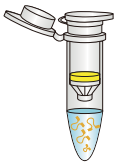
Step 1 Lysis	• Transfer 200 µl sample to a 1.5 ml microcentrifuge tube. E.g. Serum, plasma, body fluids or the supernatant of a viral infected cell culture. NOTE: If the sample is less than 200 µl, adjust the sample volume to 200 µl with PBS. • Add 400 µl of VB Lysis Buffer to the sample then mix by vortex. • Incubate at room temperature for 10 minutes.
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 20°C to 25°C)
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

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7445 Chavenelle Road • Dubuque, IA 52002
800-253-4942 • (563) 690-0484 • info@ibisci.com • **IBISCI.com**