Gentaur

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BioVision
BioVision Incorporated

Xpress Virus DNA/RNA Kit

(Cat# K1361-4, -50, -100, -300; Spin Column Based; Store at RT)

I. Introduction:

The Xpress Viral DNA/RNA Kit 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. 101-109 copies of viral DNA/RNA can be purified from 200 µl of serum within 20 min. The purified viral DNA/RNA can be used directly in QPCR and QRT PCR assays.

II. Application:

- RT PCR/PCR, QPCR, QRT PCR, Real time PCR, Real time RT PCR, Automated Fluorescent DNA Sequencing, Next Generation Sequencing (NGS)

III. Key Features:

- High Sensitivity: virus RNA/DNA can be successfully extracted and detected from as low as 10E1 copy number!
- Purify virus DNA or virus RNA in 20 min!
- Sample Volume: up to 200 µl samples of plasma, serum, body fluids, supernatant of viral cell cultures
- Spin Columns: glass fiber membrane optimized for virus DNA and virus RNA purification
- Individually packaged virus spin columns and collection tubes, certified RNAse and DNAse-free
- Elution Volume: 50 µl

IV. Sample Type:

- Up to 200 µl samples of plasma, serum, body fluids, supernatant of viral cell cultures

V. Kit Contents (Xpress Virus DNA/RNA Kit):

Components	K1361-4	K1361-50	K1361-100	K1361-300	Part Number
VB Lysis Buffer	2 ml	30 ml	60 ml	130 ml	K1361-XX-1
*AD Buffer (Add Ethanol)	0.5 ml (4 ml)	4 ml (30 ml)	8 ml (60 ml)	24 ml (180 ml)	K1361-XX-2
W1 Buffer	2 ml	30 ml	50 ml	130 ml	K1361-XX-3
**Wash Buffer (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)	50 ml (200 ml)	K1361-XX-4
RNAse-free Buffer	1 ml	6 ml	6 ml	30 ml	K1361-XX-5
VB Columns	4	50	100	300	K1361-XX-6
2 ml Collection Tubes	8	100	200	600	K1361-XX-7

**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.*

VI. User Supplied Reagents and Equipment:

- Pipettes
- Pipette tips
- PBS
- Absolute ethanol
- ddH_2O (RNAse/DNAse-free)
- Microcentrifuge tubes
- ß-mercaptoethanol
- 0.10-0.25% Trypsin

VII. Shipment and Storage:

All the reagents are shipped and stored at room temperature (15-25ºC) for up to atleast 9 months without showing any reduction in performance

VIII. Reagent Preparation and Storage Conditions:

- Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
- To prevent RNAse contamination, disposable and non-disposable plasticware and automatic pipettes should be sterile and used only for RNA procedures
- The Viral Nucleic Acid Extraction Kit II is optimized to eliminate the need for Carrier RNA and Internal Control (IC).

IX. Xpress Virus DNA/RNA Kit Protocol:

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

Step 1. Cell 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 min.

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 14-16,000g for 1 min.
- 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 14-16,000g for 1 min.
- 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 14-16,000g for 30 sec.

- 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 14-16,000g for 30 sec.
- Discard the flow-through and place the VB Column back in the 2 ml Collection Tube.
- Centrifuge at 14-16,000g for 3 min 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 min to ensure the RNAse-free Water is absorbed by the matrix.
- Centrifuge at 14-16,000g for 1 min to elute the purified nucleic acid.

X. Sensitivity:

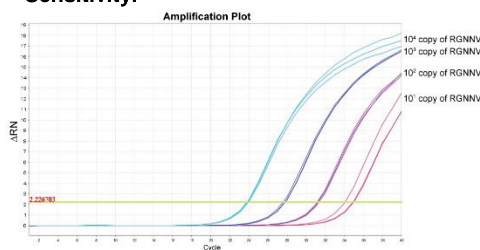

Figure 1. Virus RNA was purified from 10E1-10E4 copy number of Red Spotted Grouper Nervous Necrosis Virus (RGNNV) using the BV Virus DNA/RNA Kit II (3 replications of each copy number). The purified RNA was eluted with 30 µl RNAse-free Water. cDNA synthesis was carried out with a 10 µl aliquot of purified 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 StepOnePlusTM 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 C_t values indicate a high number of target nucleic acid in the sample.

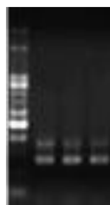

Figure 2. Hepatitis A Virus (HAV) RNA was extracted using the Viral Nucleic Acid Extraction Kit II. The purified RNA was analyzed by electrophoresis on a 1% agarose gel.
M: BV 1 Kb DNA Ladder
1: HAV from original serum
2: HAV from 10X diluted serum
3: HAV from 100X diluted serum

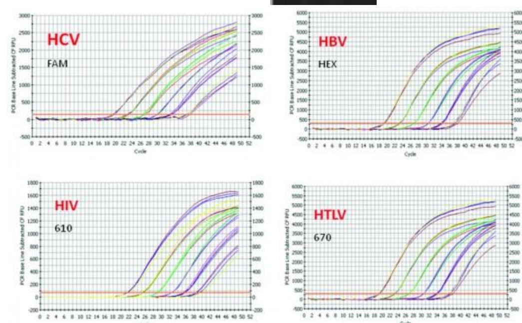

Figure 3. HBV (DNA), HCV (RNA), HIV (RNA), and HTLV (RNA) were purified from 200 µl of positive clinical serum samples using the Viral Nucleic Acid Extraction Kit II. Real time qPCR and 1-step QRT PCR reactions were then conducted using the ABI 7300 Sequence Detection System (3 replications of each copy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

XI. Related Products:

Product Name	Cat. No.	Size
Magnetic Beads Viral DNA/RNA Kit	K1409-48, -96	-48, -96 rxns
Mag-Adenovirus Purification Kit	K1459-20, -100	20, 100 preps
Mag-Lentivirus and Retrovirus Purification Kit	K1458-20, -100	20, 100 preps
Viral DNA Extraction Kit	K1446-100	100 tests

XII. General Troubleshooting Guide:

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

FOR RESEARCH USE ONLY! Not to be used on humans.