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Virus DNA/RNA Kit

(Cat# K1360-4, -50, -100, -300; Spin Column Based; Store at RT)

I. Introduction:

The 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 1 ml of serum within 60 minutes. 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:

- Purify virus DNA or virus RNA from 1 ml samples within 60 min!
- Sample Volume: 1 ml 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 1 ml plasma, serum, body fluid or the supernatant of viral infected cell cultures

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

Components	K1360-4	K1360-50	K1360-100	K1360-300	Part Number
PT Buffer	1 ml	12 ml	25 ml	70 ml	K1360-XX-1
*LS Buffer	1 ml	6 ml	12 ml	40 ml	K1360-XX-2
**Wash Buffer (Add Ethanol)	1 ml (4 ml)	5 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)	K1360-XX-3
Acid Buffer	1 ml	1 ml	1 ml	2 ml	K1360-XX-4
RNAse Free Water	1.5 ml	3 ml	6 ml	30 ml	K1360-XX-5
VB Columns	4	50	100	300	K1360-XX-6
2 ml Collection Tubes	8	50	100	300	K1360-XX-7

** 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*

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 III buffer system is optimized to eliminate the need for Carrier RNA.

IX. Virus DNA/RNA Kit 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 (please choose 1 of the following options)

- 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 min.
- Centrifuge at 14-16,000g for 15 min. 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 3X RBC lysis buffer to 200-500 µl of whole blood.
- Centrifuge at 3,000g for 15 min followed by cell ppt processing.

Step 1. Cell 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 min.

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 14-16,000g for 30 sec.
- 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 14-16,000g for 30 sec.
- Discard the flow-through.
- Add 200 µl of Wash Buffer (make sure ethanol was added) to the VB Column again.
- Centrifuge at 14-16,000g for 30 sec.
- Discard the flow-through.
- Centrifuge at 14-16,000g for 2 min to completely remove the ethanol residue.

Step 4. Wash:

- 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 min.
- Centrifuge at 14-16,000g for 1 min 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 min.
- Centrifuge at 14-16,000g for 15 min then carefully discard the supernatant.
- Dissolve ppt in 5 µl of nuclease-free ddH_2O.
- Use 1 µl for PCR or QPCR.

X. 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

XI. General Troubleshooting Guide:

Problem	Cause	Solution
Degraded RNA	Incorrect sample storage temperature	Extracted RNA should be stored at -70°C.
Low RNA Yield	Incorrect RNA elution	Make sure RNAse-free Water is added to the center of the PV Column and is absorbed completely.
Low RNA A260/A280	Incomplete wash step	Wash the PV Column with ethanol added Wash Buffer 2 times.
Sample too viscous	Sample not mixed sufficiently during lysis	Use a Thermomixer or more frequent mixing of the sample

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