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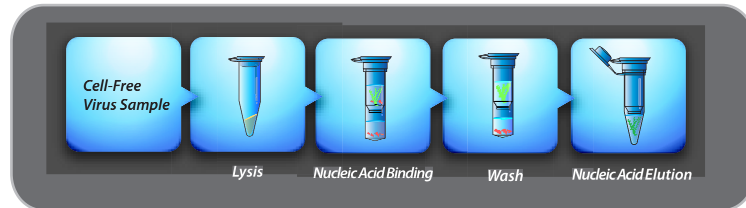

Real Biotech Corporation

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HiYield™ Viral Nucleic Acid Extraction Kit II Protocol Book

Ideal for Extracting Viral DNA/RNA from 1 ml of Cell-Free Samples

Cat. No. **YVNII50 / YVNII100 / YVNII300**

HiYield™ Viral Nucleic Acid Extraction Kit II

Cat. No. YVNII50	Cat. No. YVNII100	Cat. No. YVNII300
50 preps/ kit	100 preps/ kit	300 preps/ kit
PP Buffer: 12 ml	PP Buffer: 25 ml	PP Buffer: 70 ml
LS Buffer: 6 ml *	LS Buffer: 12 ml *	LS Buffer: 40 ml *
Wash Buffer(concentrated): 5 ml **	Wash Buffer(concentrated): 12.5 ml **	Wash Buffer(concentrated): 25 ml **
Acid Buffer: 1 ml	Acid Buffer: 1 ml	Acid Buffer: 2 ml
Release Water: 3 ml	Release Water: 6 ml	Release Water: 30 ml
VB Column: 50 pcs	VB Column: 100 pcs	VB Column: 300 pcs
2ml Collection Tube: 100 pcs	2ml Collection Tube: 200 pcs	2ml Collection Tube: 600 pcs
All components are RNase -Free	All components are RNase -Free	All components are RNase -Free

Sample: Up to 1 ml of Plasma, Serum, Body Fluid or Supernatant of Viral Infected Cell Cultures
Viruses Included: Retroviruses, Influenza, Enteroviruses, DNA Viruses etc.
Format: Spin Columns
Operation: Centrifuge or Vacuum
Operation Time: 55 Minutes

* If precipitates have formed in the LS Buffer, warm the buffer in a 37 °C water bath to dissolve.
** Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Description

HiYield™ Viral Nucleic Acid Extraction Kit II is designed for simultaneous purification of viral DNA and RNA from 1 ml of Serum, plasma, cell-culture supernatants and cell-free body fluids. This kit represents a well established membrane technology for viral nucleic acid preparation. The procedure is based on binding viral DNA/RNA to the surface of the membrane, and finally release viral DNA/RNA into the release water. The unique membrane guarantees extremely high recovery of pure viral DNA and RNA without using of phenol/chloroform extraction or alcohol precipitation. The entire procedure could be completed within 55 minutes. The so purified viral DNA/RNA can be used directly in qPCR and qRT-PCR assays.

Features

Rapid isolation of cell-free viral DNA/RNA.
Complete removed of all contaminants for reliable downstream applications.
Simple procedure.

Applications

Purified nucleic acid is ready for wide range of downstream applications, such as RT-PCR, PCR, Real-Time PCR, Real-Time RT-PCR, Automated Fluorescent DNA sequencing and many other enzymatic reactions.

Quality Control

The quality of the HiYield™ Viral Nucleic Acid Extraction Kit II is tested on a lot-to-lot basis by isolating viral DNA/RNA from a 1 ml serum sample.

Product Intended Use (Research/Clinical Application):

HiYield™ Viral Nucleic Acid Extraction Kit II is general purpose device. Real Biotech Corporation has not validated in clinical application for any particular system or organism and therefore offered no specific claims for uses in prognostics, diagnostics, blood banking etc. This device may be used in clinical diagnostics laboratory systems for molecular assays after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or local equivalents in other countries. All due care and attention should be exercised in handling this product.

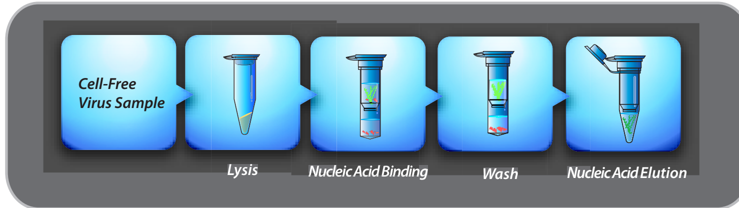
Reference: Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

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

Protocol

Additional requirements:

* 96-100% Ethanol
* 1.5 ml microcentrifuge tube (DNase and RNase free)
* Sterile, RNase-free pipet tips
* 3 M NaOAc pH 5.2
* Isopropanol



Things to do before starting:

* If precipitates have formed in the LS Buffer, warm the buffer in a 37 °C water bath to dissolve.
* Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
* Prior to using this kit, preheat the Release Water to 65 °C.

Sample Preparation

1. Concentrate the virus by adding 150 µl of PP Buffer to 1 ml of serum or plasma ,and mix well (if the sample volume is less than 1 ml, 150 µl of PP Buffer is still required). Let stand at room temperature for 30 minutes. Centrifuge at 12,000 rpm for 15 minutes.
2. Remove the supernatant and save the viral ppt (to purify genomic DNA by HIV and HTLV Proviral DNA Integration from whole blood samples, 200-500 µl of whole blood is first lysed by 3 x RBC Lysis Buffer, centrifuged at 3,000 rpm for 15 minutes, followed by cell ppt processing).

Lysis

3. Mix 100 µl of LS Buffer with 1 µl of Internal Control (short ds DNA, E3/µl) and vortex.
4. Add 100 µl of the mixture into the viral ppt, vortex and incubate at room temperature for 5 minutes.

Nucleic Acid Binding

5. Add 234 µl of absolute ethanol to the mixture from step 4 and mix by shaking 10 times.
6. Place a VB Column in a 2 ml Collection Tube and transfer the mixture to the VB column.
7. Centrifuge at 12,000 rpm for 30 seconds.
8. Discard the 2 ml Collection Tube containing the flow-through and transfer the VB Column to a new 2 ml Collection Tube.

Wash

9. Add 200 µl of Wash Buffer to the VB Column.
10. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
11. Add 200 µl of Wash Buffer to the VB Column again.
12. Centrifuge again at 12,000 rpm for 30 seconds. Discard the flow-through.
13. Centrifuge at 12,000 rpm for 2 minutes to completely remove the ethanol residue.

Nucleic Acid Elution

14. Add 50 µl of Release Buffer (preheated to 65 °C) to the center of the column matrix to release the viral DNA/RNA and Internal Control.
15. Let stand at 65 °C for 3 minutes. Centrifuge at 12,000 rpm for 1 minute to elute the purified viral DNA/RNA.

Optional Step: Nucleic Acid Concentration

a. Add 5 µl of Acid Buffer and 50 µl of Isopropanol to the eluted product, mix well and let stand at room temperature for 10 minutes. Centrifuge at 12,000 rpm for 15 minutes and carefully discard the supernatant.
b. Dissolve ppt in 5 µl of nuclease-free ddH2O.
c. Use 1 µl for PCR or qPCR.

HiYield™ Viral Nucleic Acid Extraction Kit II

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