Presto™ 96 well Viral Nucleic Acid Extraction Kit

For research use only

Sample : up to 200 µl plasma, serum, body fluid
up to 200 µl supernatant of viral infected cell cultures
Format : Presto™ Viral DNA/RNA 96 Well Binding Plates
Binding Capacity : 50 µg of DNA/RNA per well
Operation time : 60 minutes
Elution volume : 30 µl from 50 µl RNase-free water volume
60 µl from 80 µl RNase-free water volume
Dead volume : 20 µl

CERTIFICATE NO. QAIC/TW/50077

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Introduction

The Presto™ 96 well Viral Nucleic Acid Extraction Kit was designed for high-throughput purification of high-quality of viral DNA and viral RNA from cell-free samples such as serum, plasma, body fluids and the supernatant of viral infected cell cultures. Detergents and chaotropic salt are used to lyse and inactivate viruses. The efficient glass fiber in each well 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 60 minutes. The purified viral DNA/RNA can be used directly in qPCR and qRT-PCR assays.

Quality Control

The quality of Presto™ 96 well Viral Nucleic Acid Extraction Kit 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	96VR02	96VR04	96VR10
VB Lysis Buffer	30 ml x1 60 ml x1	60 ml x1 130 ml x1	30 ml x1 130 ml x3
AD Buffer[1] (Add ethanol)	12 ml (90 ml)	24 ml (180 ml)	12 ml x1 (90 ml x1) 24 ml x2 (180 ml x2)
W1 Buffer	130 ml	50 ml x1 130 ml x1	50 ml x1 130 ml x3
Wash Buffer[2] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml x1 (100 ml x1) 50 ml x2 (200 ml x2)
RNase-free Water	15 ml	30 ml	30 ml x2
Carrier RNA[3] (Add RNase-free Water)	1 mg (1 ml)	1 mg (1 ml)	1 mg (1 ml)
Presto™ Viral DNA/RNA 96 Well Binding Plate	2 pcs	4 pcs	10 pcs
96 Deep Well Plate[4]	2 pcs	2 pcs	2 pcs
0.35 ml Collection Plates	2 pcs	4 pcs	10 pcs
Adhesive Film	4 pcs	8 pcs	20 pcs

[1,2] Add absolute ethanol (see the bottle label for volume) to AD Buffer and Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[3] Add 1 ml of RNase-free Water to Carrier RNA then vortex to ensure Carrier RNA is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The Carrier RNA and RNase-free Water solution should be stored at -20ºC. Do not freeze and thaw Carrier RNA solution more than 3 times.

[4] 96 Deep Well Plates are reusable. After use, rinse the plate with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O. The plate can be autoclaved after being washed.

Caution

VB Lysis Buffer contains chaotropic salt. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask. Disposable/non-disposable glassware, plasticware and automatic pipettes should be sterile (RNase-free).

Additional Requirements:

Centrifuge with microplate buckets or vacuum manifold, additional 96 Deep Well Plates, absolute ethanol.

Presto™ 96 well Viral Nucleic Acid Extraction Kit Centrifuge Protocol

Step 1 Sample preparation	**For cell-free samples (serum, plasma, body fluids)** • For 96 samples: add **40 ml of VB Lysis Buffer** and **100 µl of Carrier RNA** into a clean 50 ml tube, mix by vortex for 10 seconds. • Add **400 µl of VB Lysis Buffer containing Carrier RNA** into each well of a clean 96 Deep Well Plate using a multichannel pipette. • Transfer 200 µl sample into each well of the 96 Deep Well Plate and mix the sample mixture by pipetting 6-8 times using a multichannel pipette. Note: If the prepared sample is less than 200 µl, adjust the sample volume to 200 µl with PBS. Careful pipetting the sample mixture to prevent cross contamination is obligatory. • Incubate the sample mixture at room temperature for 10 minutes. **For samples preserving in Geneaid SYNCStore™ STM** • Add 1 µl of Carrier RNA into each tube of SYNCstore™ STM and vortex briefly. • Transfer 600 µl of medium into each well of a clean 96 Deep Well Plate. • Proceed with viral nucleic acid binding step.
Step 2 Viral Nucleic Acid Binding	• Add **450 µl of AD Buffer (make sure absolute ethanol was added)** into each well of the 96 Deep Well Plate and mix the sample mixture by pipetting 6-8 times using a multichannel pipette. Note: Careful pipetting the sample mixture to prevent cross contamination is obligatory. • Place the **Presto™ Viral DNA/RNA 96 Well Binding Plate** on an empty **96 Deep Well Plate**. • Carefully transfer **500 µl of sample lysate** into each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate. Be careful do not wet the rims of the wells. • Centrifuge the Presto™ Viral DNA/RNA 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. • Transfer the **remaining sample lysate** into each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate. Be careful do not wet the rims of the wells. • Centrifuge the Presto™ Viral DNA/RNA 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. • Discard the flow-through then place the Presto™ Viral DNA/RNA 96 Well Binding Plate back on the 96 Deep Well Plate.
Step 3 Wash	• Add **400 µl of W1 Buffer** to each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate then centrifuge the Presto™ Viral DNA/RNA 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. • Discard the flow-through then place the Presto™ Viral DNA/RNA 96 Well Binding Plate back on the 96 Deep Well Plate. • Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate then centrifuge the Presto™ Viral DNA/RNA 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. • Discard the flow-through then place the Presto™ Viral DNA/RNA 96 Well Binding Plate back on the 96 Deep Well Plate. • Centrifuge the Presto™ Viral DNA/RNA 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 10 minutes to dry the membrane.

Step 4 Elution	• Remove the Presto™ Viral DNA/RNA 96 Well Binding Plate from the 96 Deep Well Plate then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. • Place the Presto™ Viral DNA/RNA 96 Well Binding Plate on top of a **0.35 ml Collection Plate**. • Add **50-80 µl of RNase-free Water** into the **CENTER** of each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate. • Let stand for at least 2 minutes to ensure the RNase-free water is absorbed by the membrane. • Centrifuge the Presto™ Viral DNA/RNA 96 Well Binding Plate and the 0.35 ml Collection Plate together at 3,000 x g for 5 minutes. • Seal the 0.35 ml Collection Plate with new **Adhesive Film** then store the purified viral nucleic acid at -70ºC.

Presto™ 96 well Viral Nucleic Acid Extraction Kit Vacuum Protocol

Vacuum Manifold Preparation	• Place the **Waste Tray** on the Manifold Base, place the Binding Top Plate on the manifold base, and place the **Presto™ Viral DNA/RNA 96 Well Binding Plate** in the Binding Top Plate aperture. • Attach the vacuum manifold to a vacuum source.
Step 1 Sample Preparation	**For cell-free samples (serum, plasma, body fluids)** • For 96 samples: add **40 ml of VB Lysis Buffer** and **100 µl of Carrier RNA** into a clean 50 ml tube, mix by vortex for 30 seconds. • Add **400 µl of VB Lysis Buffer containing Carrier RNA** into each well of a clean 96 Deep Well Plate using a multichannel pipette. • Transfer 200 µl sample into each well of the 96 Deep Well Plate and mix the sample mixture by pipetting 6-8 times using a multichannel pipette. Note: If the prepared sample is less than 200 µl, adjust the sample volume to 200 µl with PBS. Careful pipetting the sample mixture to prevent cross contamination is obligatory. • Incubate the sample mixture at room temperature for 10 minutes. **For samples preserving in <u>Geneaid SYNCStore™ STM</u>** • Add 1 µl of Carrier RNA into each tube of SYNCstore™ STM and vortex briefly. • Transfer 600 µl of medium into each well of a clean 96 Deep Well Plate. • Proceed with viral nucleic acid binding step.
Step 2 Viral Nucleic Acid Binding	• Add **450 µl of AD Buffer (make sure absolute ethanol was added)** into each well of the 96 Deep Well Plate and mix the sample mixture by pipetting 6-8 times using a multichannel pipette. Note: Careful pipetting the sample mixture to prevent cross contamination is obligatory. • Transfer **1 ml of the sample lysate** into each well of the **Presto™ Viral DNA/RNA 96 Well Binding Plate**. Be careful do not wet the rims of the wells. Note: Seal unused wells of the Presto™ Viral DNA/RNA 96 Well Binding Plate with Adhesive Film. • Apply vacuum at 15 inches Hg until sample passes through the Presto™ Viral DNA/RNA 96 Well Binding Plate, switch off the vacuum.

Step 3 Wash	• Add **400 µl of W1 Buffer** to each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate. • Apply vacuum at 15 inches Hg until W1 Buffer passes through the Presto™ Viral DNA/RNA 96 Well Binding Plate (approximate 10 seconds), switch off the vacuum. • Discard the flow-through and assemble the manifold again. • Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate. • Apply vacuum at 15 inches Hg until Wash Buffer passes through the Presto™ Viral DNA/RNA 96 Well Binding Plate. • Continue to apply vacuum for additional 10 minutes to dry the membrane, switch off the vacuum.
Step 4 Elution	• Remove the Presto™ Viral DNA/RNA 96 Well Binding Plate from the binding top plate aperture and blot the nozzles on a clean absorbent paper towel to remove residual ethanol. • Remove the Waste Tray from the manifold base then place the collection plate spacer on the manifold base. • Place a **0.35 ml collection plate** on top of the collection plate spacer. • Place the binding top plate back on the manifold base then place the Presto™ Viral DNA/RNA 96 Well Binding Plate back in the binding top plate aperture. • Add **50-80 µl of RNase-free water** into the **CENTER** of each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate. • Let stand for at least 2 minutes to ensure the RNase-free water is absorbed by the membrane then apply vacuum at 15 inches Hg for 5 minutes. • Seal the 0.35 ml Collection Plate with Adhesive Film then store the purified viral nucleic acid at -70ºC.

Troubleshooting

Problem	Cause	Solution
Low yield	A. Sample lysis or homogenization was incomplete B. Incorrect buffer preparation C. Incorrect RNA elution	A. Starting material should not contain too many cells. B. Absolute ethanol should be added into AD Buffer and Wash Buffer prior to use. C. Make sure RNase-free Water is added to the center of each well of the Presto™ Viral DNA/RNA 96 Well Binding Plate and is absorbed completely.
Eluted RNA does not perform well in downstream applications	A. Residual ethanol contamination	A. Following the wash step, dry the Presto™ Viral DNA/RNA 96 Well Binding Plate with additional centrifugation at 3,000 x g or with additional vacuum for 10 minutes to ensure the membrane is completely dry.