

# Virus Total Nucleic Acid Isolation Kit

For research use only

**Sample:** serum, plasma, body fluids and the supernatant of viral infected cell cultures

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## Introduction

The Virus Total Nucleic Acid Isolation Kit provides a fast, simple, and cost-effective method for isolation of viral DNA/RNA from cell-free samples such as serum, plasma, body fluids and the supernatant of viral infected cell cultures. Its unique buffer system efficiently lyses cells and degrades proteins, allowing for nucleic acids to bind to the glass fiber matrix of the columns easily. Contaminants such as salts, metabolites and soluble macromolecular cellular components are removed through the Wash step. Phenol extraction and ethanol precipitation are not required, and high-quality Nucleic Acids are eluted with RNase-free elution buffer. Viral DNA/RNA isolated with FairBiotech's Virus Total Nucleic Acid Isolation Kits is suitable for a variety of routine applications, including Real-time PCR/RT-PCR, Automated Fluorescent DNA Sequencing, PCR, and other enzymatic reactions. The entire procedure can be completed within 15-20 minutes.

## Kit Contents

| Catalog No.                | VN0004                | VN0100           | VN0200            | VN0300               |
|----------------------------|-----------------------|------------------|-------------------|----------------------|
| V1 Buffer                  | 1.5 ml                | 45 ml            | 85 ml             | 125 ml               |
| V2 Buffer<br>(Add Ethanol) | 220 µl<br>(1650 µl)   | 6 ml<br>(45 ml)  | 12 ml<br>(90 ml)  | 16 ml<br>(120 ml)    |
| W1 Buffer                  | 2 ml                  | 45 ml            | 85 ml             | 125 ml               |
| W2 Buffer<br>(Add Ethanol) | 300µl×2<br>(1.2 ml)×2 | 15 ml<br>(60 ml) | 25 ml<br>(100 ml) | 25 ml×2<br>(100ml)×2 |
| RNase-free EL Buffer       | 1 ml                  | 10 ml            | 20 ml             | 30 ml                |
| VN Columns                 | 4 pcs                 | 100 pcs          | 200 pcs           | 300 pcs              |
| Collection Tubes           | 4 pcs                 | 100 pcs          | 200 pcs           | 300 pcs              |

## NOTE

- ★ Add ethanol (96–100%) to Buffer V2 and W2, **shake before use** (see bottle label for volume).
- ★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
- ★ Buffers V1 and W1 contain irritants. Wear gloves when handling these buffers.

## Virus Total Nucleic Acid Isolation Kit Protocol

### Step 1 Lysis

- ◆ Transfer up to 200 µl of virus sample into a 1.5 ml microcentrifuge tube and add **400 µl of V1 Buffer**. (If the sample is less than 200 µl, adjust the sample volume to 200 µl with PBS)
- ◆ Mix well and let stand at room temperature for 10 minutes.
- ◆ #Pre-heat the Elution Buffer to 75°C for Step 4 DNA Elution.

### Step 2 Nucleic Acid Binding

- ◆ Add **450 µl of V2 Buffer** (ethanol added) to the sample lysate and shake vigorously.
- ◆ Place a **VN Column** in a **Collection Tube**. Transfer 700 µl of the lysate mixture to the **VN Column**.
- ◆ Centrifuge at 16,000 x g for 1 minute. Discard the flow-through and place the **VN Column** back in the **Collection Tube**.
- ◆ Transfer the remaining lysate mixture to the **VN Column**.
- ◆ Centrifuge at 16,000 x g for 1 minute. Discard the flow-through and place the **VN Column** back in the **Collection Tube**.

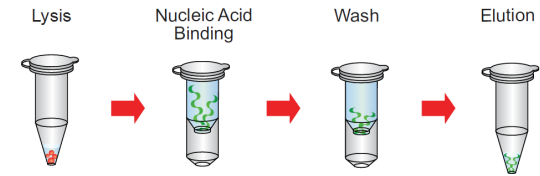
### Step 3 Wash

- ◆ Add 400 µl of **W1 Buffer** into the **VN Column**. Centrifuge at 16,000 x g for 30 seconds. Discard the flow-through and place the **VN column** back into the **Collection tube**.

- ◆ Add 600 µl of **W2 Buffer** (ethanol added) into the **VN Column**. Centrifuge at 16,000 x g for 30 seconds. Discard the flow-through and place the **VN column** back in the **Collection tube**.
- ◆ Centrifuge at 16,000 x g again for 2 minutes to remove residual **W2 Buffer**.

### Step 4 Elution

- ◆ Place the **VN column** in a clean 1.5 ml microcentrifuge tube (DNase and RNase free).
- ◆ Add **50-100 µl of Pre-Heated EL Buffer** or RNase-free water (pH is between 7.0 and 8.5) to the center of each **VN column**, let stand for 2 min, and centrifuge at 14,000 x g for 2 min.



## Troubleshooting

| Problem  | Cause  | Solution  |
|--|--|---|
| Poor performance of RNA in downstream applications | Interference of the residual ethanol                                   | Be sure to remove Buffers V2 and W2 completely.   |
| Low yields   | Insufficient performance of the elution buffer during the elution step | Remove the residual buffers during the wash steps completely. These residual buffers decrease the efficiency of the following elution steps.                            |
|  | Incomplete lysis   | Check the incubation time of the Lysis Step.  |
|  | Viral nucleic acid remains on the column                               | Repeat the Elution Step with the eluant. Extend the time EL Buffer or RNase-free water stays on column centers from Step 4 from 2 to 5 minutes prior to centrifugation. |
| Degraded RNA                                       | Source   | Do not freeze and thaw sample more than once. Increase the virus concentration in the sample.   |
|  | RNase contamination  | Be sure not to introduce RNase during the procedure. Check buffers for the RNase contamination.   |