Virus Nucleic Acid Isolation Kit

Cat No. PDC03-0100
Size: 100 Reactions

Sample: Up to 300 µl of the whole blood or Up to 200 µl of virus sample
Format: Reagent and mini spin column **Operation time:** 20 minutes
Sample material: Serum, plasma, body fluids **Elution volume:** 50 µl

Description

The **Virus Nucleic Acid Isolation Kit** provides a fast, simple, and cost-effective method for the isolation of viral DNA/RNA from cell-free samples such as serum, plasma, body fluids and the supernatant of virus-infected cell cultures. Its unique buffer system will efficiently lyse cells and degrade protein, allowing for the nucleic acid to be easily bound by the glass fiber matrix of the column. Contaminants such as salts, metabolites and soluble macromolecular cellular components are removed in the Wash Step. The phenol extraction and ethanol precipitation are not required, and the high-quality nucleic acid is eluted in the RNase-free elution buffer. The viral DNA/RNA isolated with the Total Nucleic Acid Isolation Kit (Virus) is suitable for a variety of routine applications, including the 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

Contents	PDC03-0100	PDC03-0100S
Buffer V1	45 ml	1.5 ml
Buffer V2 (Add ethanol)	6 ml (45ml)	220 µl (1650 µl)
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.5 ml x2)
Buffer RE	10 ml	1 ml
Column VN	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

Quality Control

The quality of the Virus Nucleic Acid Isolation Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- 1.5 ml Microcentrifuge tubes (DNase and RNase free)
- PBS (Phosphate Buffered Saline
- Absolute ethanol (96~100%)

Virus Nucleic Acid Isolation Kit Protocol

Step 1 Lysis

1. Transfer up to 200 µl of the virus sample into a 1.5 ml microcentrifuge tube and add 400 µl of the Buffer V1. (If the sample is less than 200 µl, adjust the sample volume to 200 µl with the PBS)
2. Mix well and let it stand at the room temperature for 10 minutes.

Step 2 Nucleic Acid Binding

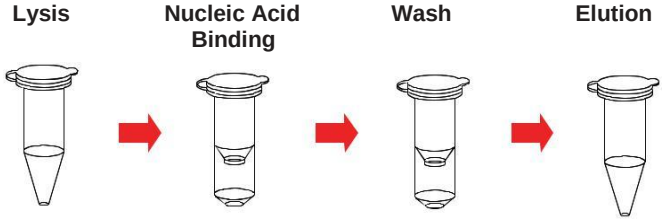
1. Add 450 µl of the Buffer V2 (ethanol added) to the sample lysate and shake vigorously.
2. Place a Column VN in a 2 ml Collection Tube.
3. Transfer 700 µl of the lysate mixture into the Column VN.
4. Centrifuge at 16,000 x g for 1 minute.
5. Discard the flow-through and place the Column VN back in the same Collection Tube.
6. Transfer the remaining lysate mixture to the Column VN.
7. Centrifuge at 16,000 x g for 1 minute.
8. Discard the flow-through and place the Column VN back in the same Collection Tube.

Step 3 Wash

1. Add 400 µl of the Buffer W1 into the Column VN.
2. Centrifuge at 16,000 x g for 30 seconds.
3. Discard the flow-through and place the Column VN back into the same Collection tube.
4. Add 600 µl of Buffer W2 (ethanol added) into the Column VN.
5. Centrifuge at 16,000 x g for 30 seconds.
6. Discard the flow-through and place the Column VN back into the same Collection tube.
7. Centrifuge at 16,000 x g again for 2 minutes to remove the residual Buffer W2.

Step 4 Elution

1. Place the Column VN in a clean 1.5 ml microcentrifuge tube (DNase and RNase free).
2. Add 50 µl Buffer RE or RNase-free water (pH is between 7.0 and 8.5) to the center of each Column VN, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying the viral RNA and DNA with the kit.

Problem	Cause	Solution
Low yields	Insufficient performance of the elution buffer during the elution step	Remove the residual buffers during the wash steps completely. The remaining 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. Incubate the column for 5 min with water prior to centrifugation.
Poor performance of RNA in downstream applications	Interference of the residual ethanol	Be sure to remove the entire Buffer V2 and W2 completely.
Degraded RNA	Source	Do not freeze and thaw sample more than once. Increase the viral concentration in the sample.
	RNase contamination	Ensure not to introduce RNase during the procedure. Check buffers for the RNase contamination.

Caution

- Add 45 ml and 60 ml of the ethanol (96–100%) to the Buffer V2 and W2, and shake before use.
- Check the Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- The Buffers V1 and W1 contain irritants. Wear gloves when handling these buffers.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.