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Total Nucleic Acid Isolation Kit-Virus

Cat. Number: TDRNAV

Storage: Store at Room Temperature.

Sample: Up to 200ul of serum, plasma, body fluids and the supernatant of viral infected cell cultures

Kit Contents

Total Nucleic Acid Isolation Kit-Virus	100 Reactions
VN Column with 2 mL Collection Tube	100pcs
Resuspension Buffer	45mL
Lysis Buffer (add Ethanol)	6mL (45mL)
Wash Buffer 1	45mL
Wash Buffer 2 (add Ethanol)	15mL (60mL)
Elution Buffer	10mL
This Datasheet	1 copy

Note:

- Add ethanol (96-100%) to Lysis Buffer and Wash Buffer 2, shake before use (see bottle label for volume).
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming to 37°C.
- Resuspension Buffer and Wash Buffer 1 contain irritants. Wear gloves when handling these buffers.

Additional requirements

- Absolute ethanol
- PBS (Phosphate Buffered Saline)
- Microcentrifuge tubes (DNase and RNase free)

Description

The Total Nucleic Acid Isolation Kit-Virus 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 Total Nucleic Acid Isolation Kit-Virus 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.

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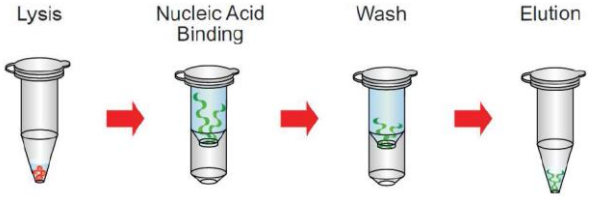
Procedure for Preparation of Total Nucleic Acid Isolation



Step 1. Lysis

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

Step 2. Nucleic Acid Binding

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

1. Add 400 µl of Wash Buffer 1 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.
2. Add 600 µl of Wash Buffer 2 (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.
3. Centrifuge at 16,000 x g again for 2 minutes to remove residual Wash Buffer 2 Buffer.

Step 4. Elution

1. Place the VN column in a clean 1.5 ml microcentrifuge tube (DNase and RNase free).
2. Add 50-100 µl of Pre-heated Elution 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

Problems	Possible Causes	Suggestions
Poor performance of RNA in downstream applications	Interference of the residual ethanol	Be sure to remove Lysis Buffer and Wash Buffer 2 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 Elution 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.