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Sin Nombre Virus RT-PCR Detection Kit

Product Insert

Product #51900

Pathogen Information

Sin nombre virus is a member of the Hantavirus genus in the Bunyaviridae family. Hantavirus pulmonary syndrome (HPS), caused by Sin nombre virus, is a rare disease but is frequently fatal, possessing a 50% mortality rate. The flu-like symptoms are very severe. The virus is endemic to rodents, so it is spread to humans through contact with rodent droppings and urine. This can be accomplished by direct contact with excreta, aerosolization of urine, especially due to sweeping in rodent-infested homes, or contact with fomites, such as contaminated blankets or food storage areas. There is no vaccine against Sin nombre virus at this time.

Principle of the Test

Norgen's Sin Nombre Virus (SNV) RT-PCR Detection Kit constituents a ready-to-use system for the isolation and detection of SNV using end-point one step RT-PCR. The kit first allows for the isolation of virus RNA from blood using spin-column chromatography based on Norgen's proprietary resin. The RNA viroid is isolated free from inhibitors, and can then be used as the template in a one step RT-PCR reaction for SNV detection using the provided SNV Master Mix. The SNV Mastermix contains reagents and enzymes for the specific amplification of a 341 bp region of the virus genome. In addition, Norgen's SNV RT-PCR Detection Kit contains a second Mastermix, the Control 2X RT-PCR Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate RT-PCR reaction with the use of the provided *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Lysis Solution	10 mL
Wash Solution	11 mL
Elution Solution	2 mL
Mini Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
SNV 2x RT-PCR Master Mix	0.35 mL
Control 2x RT-PCR Mastermix	0.35 mL
Isolation Control (IsoC)*[a]	0.3 mL
SNV Positive Control (PosC)*[b]	0.1 mL
Nuclease Free-Water	1.25 mL
Norgen's DNA Marker	0.1 mL
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** IsoC = Isolation Control ; PosC= Positive Control*
[a] The isolation control is a RNA transcript product.
[b] The positive control is SNV RNA transcript

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 96 – 100% ethanol
- Thermocycler and or Real-Time PCR System
- Micropipettes with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
- Vortex
- Sterile, nuclease-free aerosol-barrier micropipettor tips
- Microcentrifuge tube rack
- Disposable latex gloves

Storage Conditions and Product Stability

- The Positive Control (**SNV *PosC,*** red cap) and Isolation Control (***IsoC*****,** orange cap) should be stored at -70°C. If needed, make aliquots of the controls according to the volume used in the protocol (10 µL of **SNV *PosC*** or 10 µL of ***IsoC***) prior to freezing.
- The **SNV 2X RT-PCR Mastermix** (green cap) and the **Control 2X RT-PCR Mastermix** (yellow cap) should be stored at -20°C upon receipt (-70°C for long-term). Make appropriate aliquots and store at -20°C if needed.
- All other kit components may be stored at room temperature
- The **SNV 2X RT-PCR Mastermix** and the **Control 2X RT-PCR Mastermix**, Positive Control and Isolation Control should not undergo repeated freeze-thaw (a maximum freeze-thaw of three times).
- For RT-PCR
    - Allow reagents to thaw at room temperature prior to use
    - When thawed, mix the components and centrifuge briefly
    - Work quickly on ice
    - After addition of RT-PCR Mastermix use within one hour

General Precautions

The user should exercise the following precautions when using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's SNV RT-PCR Detection Kit, including the SNV 2x RT-PCR Master Mix, Control 2X RT-PCR Mastermix, Isolation Control and SNV Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's SNV RT-PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Disclaimers
The **Lysis Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Safety Information
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only.
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water.
- Clean all surfaces with commercially available RNase decontamination solutions.
- When working with purified RNA samples, ensure that they remain on ice during downstream applications.

Protocol

A. SNV Total RNA Isolation

Important Notes Prior to Beginning Protocol:

- Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed microcentrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution** by adding 25 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution. This will give a final volume of 36 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is important to work quickly during this procedure. **Isolation Control (*IsoC*)**
    - An Isolation Control (*IsoC*) is supplied. This allows the user to control the RNA isolation procedure. For this assay, add the Isolation Control *(IsoC)* to the lysate during the isolation procedure

- The Isolation Control *(IsoC)* must not be added to the sample material directly.
- Do not freeze and thaw the Isolation Control *(IsoC)* more than 2 times.
- The SNV Isolation Control *(IsoC)* must be kept on ice at all times during the isolation procedure.

- The RT-PCR components of the SNV RT-PCR Detection Kit should remain at -20^{o}C until RNA is extracted and ready for RT-PCR amplification.
- It is recommended that no more than 100 µL of blood be used in order to prevent clogging of the column.
- We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA or heparin as the anti-coagulant.
- It is important to work quickly during this procedure.

1. Lysate Preparation

a. Add 350 µL of the **Lysis Solution** to an RNase-free microcentrifuge tube.
b. Add up to 100 µL of blood. Vortex for 10 seconds to mix.
c. Add 10 µL of the Isolation Control (***IsoC***) to the lysate. Vortex for 10 seconds to mix.
d. Add 250 µL of 96-100% ethanol to the lysate. Vortex for 10 seconds to mix.
e. Proceed to RNA Isolation (Step B).

2. Binding RNA to Column

a. Apply up to 600 µL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **14000 × g (~14,000 RPM**). Discard the flowthrough and reassemble the spin column with the collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

b. Depending on your lysate volume, repeat step **2a** if necessary.

3. Column Wash

a. Apply 400 µL of **Wash Solution** to the column and centrifuge for 1 minute.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **3a** and **3b** to wash column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **Elution Buffer** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **14,000 x g (~14,000 RPM)** for 1 additional minute.
d. The purified RNA sample could be used immediately for RT-PCR as described below. It is recommended that samples be placed at -70°C for long term storage.

B. SNV RT-PCR Assay Preparation

Notes:

- Before use, suitable amounts of all RT-PCR components should be completely thawed at room temperature, gently vortexed and centrifuged briefly.
- The amount of SNV 2X RT-PCR Master Mix provided is enough for up to 32 RT-PCR reactions (24 sample RT-PCR, 4 positive control RT-PCR and 4 no template control RT-PCR).
- For each sample, one RT-PCR reaction using the **SNV 2X RT-PCR Mastermix** and one RT-PCR reaction using **Control 2X RT-PCR Mastermix** should be set up in order to have a proper interpretation of the results.
- For every RT-PCR run, one reaction containing SNV Positive Control (**SNV *PosC***) and one reaction as no template control (*Nuclease Free-Water*) must be included for proper interpretation of results.
- The recommended minimum number of RNA samples tested per RT-PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of SNV Limit of Detection.

1. Prepare the RT-PCR reaction for sample detection (Set #1, using **SNV 2X RT-PCR Mastermix**) and the RT-PCR reaction for control detection (Set #2, using **Control 2X RT-PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample RNA to be used is 1 - 2 µL. Ensure that one SNV detection reaction and one control reaction is prepared for each RNA sample. Adjust the final volume of the RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. RT-PCR Assay Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
SNV 2X RT-PCR Master Mix Or Control 2X RT-PCR Master Mix	**10 µL**
Sample RNA	**2 µL**
Nuclease-Free Water	**8 µL**
Total Volume	**20 µL**

2. For every RT-PCR run, prepare **one** positive control RT-PCR as shown in Table 2 below:

Table 2. RT-PCR Positive Control Preparation

RT-PCR Components	Volume Per RT- PCR Reaction
SNV 2X RT-PCR Master Mix Or Control 2X RT-PCR Master Mix	**10 µL**
SNV *Positive Control (PosC)*	**10 µL**
Total Volume	**20 µL**

3. For every RT- PCR run, prepare **one** no template control RT-PCR as shown in Table 3 below:

Table 3. RT-PCR Negative Control Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
SNV 2X RT- PCR Master Mix Or Control 2X RT-PCR Master Mix	**10 µL**
Nuclease-Free Water	**10 µL**
Total Volume	**20 µL**

Therefore, at a minimum, each RT-PCR run will contain 6 separate RT-PCR reactions.

C. SNV One Step RT- PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step RT-PCR.

Table 4. SNV Assay Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**50°C**	**30 min**
Cycle 2	**Step 1**	**95°C**	**3 min**
Cycle 3 (40x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**63°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 4	**Step 1**	**72°C**	**5 min**
Cycle 5	**Step 1**	**4°C**	**∞**

D. SNV One Step RT- PCR Assay Results Interpretation

1. For the analysis of the RT-PCR data, the entire 15-20 µL RT-PCR Reaction should be loaded on a 1X TAE 1.5% Agarose RNA gel along with 10 µL of Norgen's RNA Marker (provided).
2. The RT-PCR products should be resolved on the 1X TAE 1.5% Agarose gel at 150V for 20 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

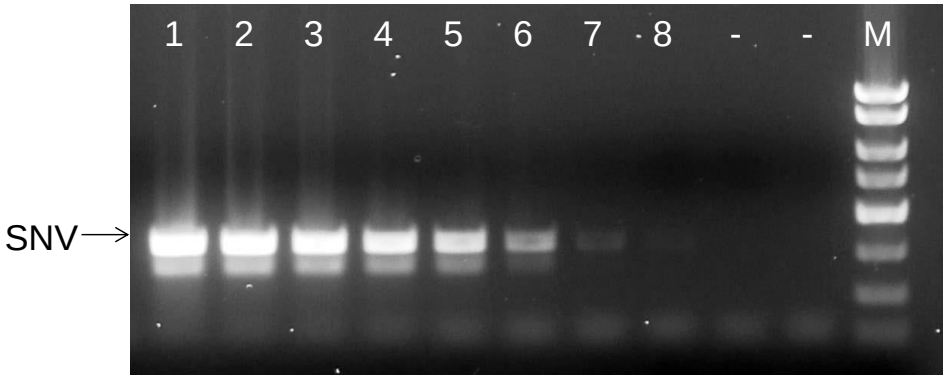

Figure 1: A representative 1X TAE 1.5% agarose gel showing the amplification of SNV serially diluted (lane 1 to 8). The size of the SNV target amplicon corresponds to 341 bp as represented by the provided DNA Marker (M). Negative control is indicated as (-).

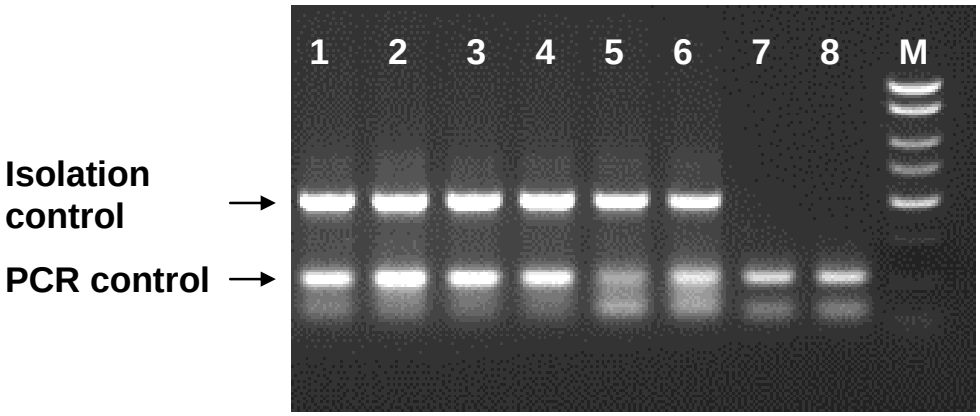

Figure 2: A representative 1X TAE 1.5% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **Control 2X RT-PCR Mastermix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 6 showed detection of both Isolation Control and PCR Control, suggesting that the RNA isolation as well as the RT-PCR reaction was successful. Lane 7 and 8 showed only the detection of PCR Control suggesting that while the RT-PCR was successful, the isolation failed to recover even the spiked-in Isolation control.

Table 5. Interpretation of One Step RT-PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	SNV Target Band (341 bp)	***IsoC* Band (499 bp)**	***PCRC* Band (150 bp)**	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

****** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

E. SNV RT-PCR Assay Specificity and Sensitivity

- The specificity of Norgen's SNV RT-PCR Detection Kit is first and foremost ensured by the selection of the SNV-specific primers, as well as the selection of stringent reaction conditions. The SNV primers were checked for possible homologies to human viruses in GenBank published sequences by sequence comparison analysis and published SNV strains.

F. Linear Range

- The linear range of Norgen's SNV RT-PCR Detection Kit was determined by analysing a dilution series of a SNV quantification standards ranging from 8 fg to 8 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen's SNV RT-PCR Detection Kit on a 1X TAE 1.5% agarose gel.
- The linear range of Norgen's SNV RT-PCR Detection Kit has been determined to cover concentrations from 8 fg to 8 pg.
- Under the conditions of the Norgen's SNV RNA Isolation procedure, Norgen's SNV RT-PCR Detection Kit covers a linear range from 1000 copies to 1 x 10^9 copies.

Frequently Asked Questions

1. How many samples should be included per RT-PCR run?

- Norgen's SNV RT-PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time.

2. How can I interpret my results if neither the SNV RT-PCR control nor the Isolation Control (*IsoC*) amplifies?

- If neither the SNV RT-PCR control nor the SNV Isolation Control (*IsoC*) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the problem has occurred during the setup of the RT-PCR assay reaction.

3. How should it be interpreted if only the SNV RT-PCR control showed amplification but neither the SNV target nor the SNV Isolation control amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the Isolation Control (*IsoC*) was amplified in a sample?

- The sample tested can be considered as SNV negative.

5. How should it be interpreted if the SNV RT-PCR control and the SNV target showed amplification in a sample?

- The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. How should it be interpreted if only the SNV target and the SNV RT-PCR control were amplified in a sample?

- The sample tested can be considered as SNV positive.

7. How should it be interpreted if only the SNV target was amplified in a sample?

- The sample tested should be considered as SNV positive. At high SNV input, the SNV amplicon will be predominant and thus the SNV RT-PCR control as well as the SNV Isolation control may not amplify as they compete for RT-PCR resources.

8. How should it be interpreted if only the SNV RT-PCR control and the Isolation control showed amplification in a sample?

- The sample tested can be considered negative

9. What if I forgot to do a dry spin after my third wash?

- Your first RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your first elution and it may interfere with the RT-PCR detection, as ethanol is known to be a PCR inhibitor.

10. What if I forgot to add the Isolation Control (*IsoC*) during the isolation?

- It is recommended that the isolation is repeated.

Related Products	Product #
Lysis Solution (100 mL)	25806
Plant RNA/DNA Purification Kit	24400
Plant/Fungi RNA Purification Kit	25800
Viroid RNA Purification Kit	32800
Bacterial Genomic RNA Isolation Kit	17900

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Sin Nombre Virus (SNV)) RT-PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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