

## HLVd/LCV/CanCV TaqMan RT-PCR Kit

Product# TM69950

## Product Insert

### Intended Use

Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit is designed for the detection of Hop Latent Viroid, Lettuce Chlorotic Virus, and Cannabis Cryptic Virus in a single PCR reaction. This kit is designed for research use only and not for use in diagnostic procedures.

### Background Information

Hop Latent Viroid (HLVd) is a single-stranded, circular infectious RNA of 256 nucleotides. HLVd infection occurs worldwide in members of the Cannabaceae family, including *Cannabis* and *Humulus* species. HLVd can be very deleterious to production in certain cultivars. Quantitative changes in some secondary metabolites in the secretory glands forming lupulin are induced by HLVd infection, indicating that the viroid is not truly latent. Hop Latent Viroid infection depends on the metabolism of the host plant, and its replication and pathogenesis are mediated by its structural features and its ability to interact with cellular factors.

Lettuce Chlorosis Virus (LCV) is a single-stranded RNA of ~8500 nucleotides. It has been found to infect many species, some notable hosts include *Cannabis* and *Lactuca* genera. Common symptoms include interveinal chlorosis, brittleness, yellowing and sometimes necrosis. Infection of LCV reduces the yield of the host crop and can lead to reduced cannabinoid potency in *Cannabis*.

Cannabis Cryptic Virus (CanCV) is a double-stranded RNA virus containing 2 molecules of dsRNA. RNA1 consists of 2420 nucleotides and RNA2 consists of 2289 nucleotides. It has been found to infect plant species of the Cannabaceae. The effects of CanCV on its hosts are not yet well understood, and it is frequently found in asymptomatic plants. More research is needed to understand the host-pathogen relationship and how this affects the growth and yield of its hosts.

### Product Description

The detection of HLVd, LCV, and CanCV specific RNA is based on TaqMan one-step RT-PCR providing a simple, reliable and rapid result for the detection of HLVd, LCV, and CanCV infection. Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. This kit contains Master Mix for the target and PCR control detection, Primer & Probe Mix, as well as a positive control and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.

This Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad CFX96 Touch™ Real-Time PCR Detection System
- QuantStudio™ 7 Pro Real-Time PCR System

### Kit Components

| Component                              | Product # TM69950 (100 Reactions) |
|--|-----------------------------------|
| MDx TaqMan 2X RT-PCR Master Mix        | 2 x 700 µL                        |
| HLVd/LCV/CanCV Primer & Probe Mix      | 280 µL                            |
| HLVd/LCV/CanCV Positive Control        | 150 µL                            |
| Nuclease-Free Water (Negative control) | 1.25 mL                           |
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### **Storage Conditions and Product Stability**

- All kit components should be stored at -20°C upon arrival
- Repeated thawing and freezing (> 2 x) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.
- All kit components can be stored for 1 year after the date of production without showing any reduction in performance.

### **Customer-Supplied Reagents and Equipment**

- Appropriate Real-Time PCR Instrument with FAM, Cy5, Tex615 and HEX filter channels
- RNA Purification Kit
  - The kit is compatible with all RNA purification kits that yield high quality, inhibitor-free RNA
  - **Recommended Purification Kit:** Norgen's Plant/Fungi Total RNA Purification Kit (Cat. 25800, 31900, 31350)
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- PCR reaction preparation station (Optional)

### **Quality Control**

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

### **Warnings and Precautions**

- Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit is intended for research purposes only. It is not intended for diagnostic use.
- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- 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.
- Dispose of unused kit reagents and specimens according to local, provincial or federal regulations.

- Do not substitute or mix reagents from different kit lots or from other manufacturers. Do not use components of the kit that have been stored for more than 1 year.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of any of the target genomes covered by the primers in this kit may result in failure to detect the presence of the pathogen.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.
- Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

## Instructions for Use

### A. Sample Preparation

Purified RNA is the starting material for Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit. The quality of the RNA template will have a major impact on the performance of the target pathogen detection test. The user must ensure that the method used for RNA purification is compatible with TaqMan One-Step RT-PCR. We recommend the use of Norgen's **Plant/Fungi Total RNA Purification Kit (Cat. 25800, 31900, 31350)**. Norgen's Plant/Fungi Total RNA Purification Kit has been fully validated with Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit.

If using a different spin column-based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 3 minutes at 20,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the RNA. This will help to prevent the carry-over of any ethanol into the purified RNA, as ethanol is known to be a strong inhibitor of PCR. **Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the RNA.**

### B. TaqMan RT-PCR Assay Preparation

#### Notes:

- Before use, suitable amounts of all TaqMan RT-PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- The amount of MDx TaqMan 2X RT-PCR Master Mix provided is enough for up to 128 RT-PCR reactions (96 sample PCR, 16 positive control PCR and 16 no template control PCR).
- For every TaqMan One-step RT-PCR run, one reaction containing HLVd/LCV/CanCV Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of RNA samples tested per TaqMan One-step RT-PCR run is 6.
- To avoid any contamination while preparing the TaqMan One-step RT-PCR assay, follow the order outlined in Tables 1, 2 and 3 below to prepare the Negative Control, Detection Assay and Positive Control:
  1. Prepare the RT-PCR Negative Control (Table 1)
  2. Prepare the RT-PCR HLVd/LCV/CanCV Assay (Table 2)
  3. Prepare the RT-PCR Positive Control (Table 3)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) MDx TaqMan 2X PCR Master Mix; 3) Primer & Probe Mix; and 4) the Sample RNA or Positive Control).

1. For each TaqMan One-step RT-PCR set, prepare one no template control PCR as shown in Table 1 below:

**Table 1. TaqMan One-step RT-PCR Negative Control Preparation**

| RT-PCR Components                 | Target detection (with MDx TaqMan 2x RT-PCR Master Mix) |
|-----------------------------------|---|
| Nuclease-Free Water               | 8 $\mu$ L   |
| MDx TaqMan 2X RT-PCR Master Mix   | 10 $\mu$ L  |
| HLVd/LCV/CanCV Primer & Probe Mix | 2 $\mu$ L   |
| Total Volume                      | 20 $\mu$ L  |

2. Prepare the One-step RT-PCR reaction for sample detection as shown in Table 2 below.

**Table 2. TaqMan One-step RT-PCR HLVd Assay Preparation**

| RT-PCR Components                 | Target detection (with MDx TaqMan 2x RT-PCR Master Mix) |
|-----------------------------------|---|
| Nuclease-Free Water               | 5 $\mu$ L   |
| MDx TaqMan 2X RT-PCR Master Mix   | 10 $\mu$ L  |
| HLVd/LCV/CanCV Primer & Probe Mix | 2 $\mu$ L   |
| Sample RNA*                       | 3 $\mu$ L   |
| Total Volume                      | 20 $\mu$ L  |

\* The recommended amount of sample RNA to be used is 3  $\mu$ L. However, a volume between 1 and 5  $\mu$ L of sample RNA may be used as template. Adjust the final volume of the RT-PCR reaction to 20  $\mu$ L using the Nuclease-Free Water provided.

3. For each RT-PCR set, prepare **one** positive control RT-PCR as shown in Table 3 below:

**Table 3. TaqMan One-step RT-PCR Positive Control Preparation**

| RT-PCR Components                      | Target detection (with MDx TaqMan 2x RT-PCR Master Mix) |
|--|---|
| MDx TaqMan 2X RT-PCR Master Mix        | 10 $\mu$ L  |
| HLVd/LCV/CanCV Primer & Probe Mix      | 2 $\mu$ L   |
| HLVd/LCV/CanCV Positive Control (PosC) | 8 $\mu$ L   |
| Total Volume                           | 20 $\mu$ L  |

## C. HLVD TaqMan One-Step RT-PCR Assay Programming

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

**Table 4. HLVD/LCV/CanCV TaqMan One-Step RT-PCR Program**

| One Step RT-PCR Cycle | Step   | Temperature | Duration |
|-----------------------|--------|-------------|----------|
| <i>Cycle 1</i>        | Step 1 | 50°C        | 30 min   |
| <i>Cycle 2</i>        | Step 1 | 95°C        | 3 min    |
| <i>Cycle 3 (40x)</i>  | Step 1 | 95°C        | 15 sec   |
|                       | Step 2 | 60°C        | 30 sec   |

**Table 5. Channel selection for specified platforms.**

| HLVd  | LCV                    | CanCV | Internal Control | Platform  |
|-------|------------------------|-------|------------------|---|
| FAM   | Texas 615 / *Texas Red | Cy5   | HEX              | BioRad CFX96 Touch™ Real-Time PCR Detection System, |
| FAM   | JUN/ *ROX              | Cy5   | VIC              | QuantStudio™  |
| Green | Orange                 | Red   | Yellow           | Qiagen Rotor-Gene Q                                 |

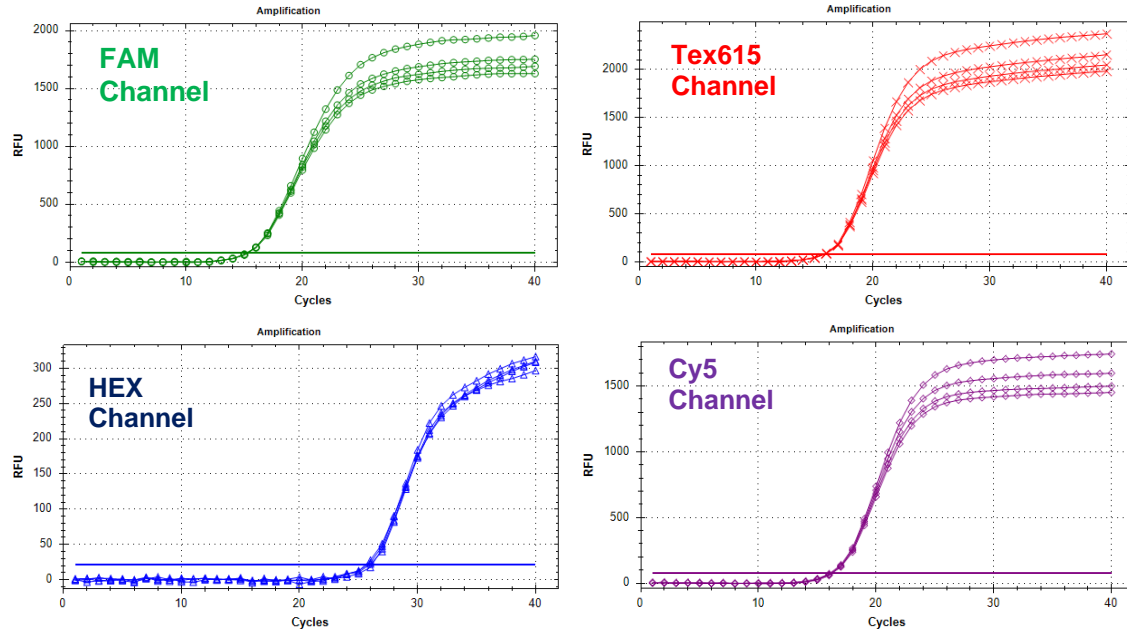
\*Note: When referencing two channels, use the first listed channel primarily, and if it not available on the model, use the second.

## D. HLVD TaqMan One-Step RT-PCR Assay Interpretation

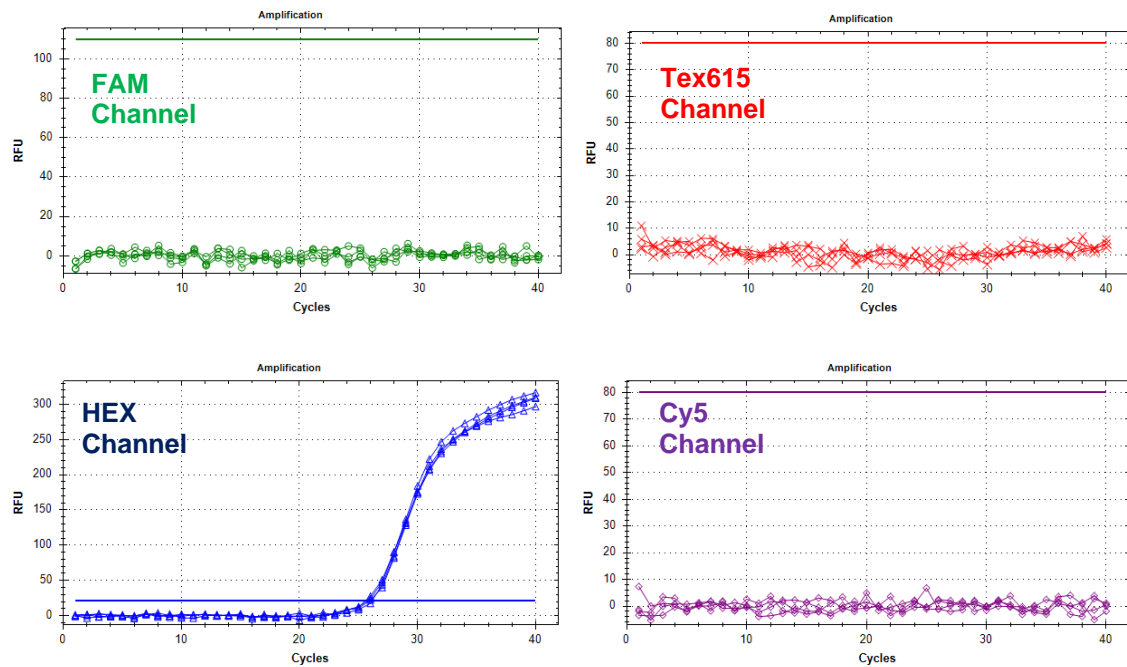
**Table 6. Interpretation of Assay Results**

| FAM (HLVd detection) | Tex615 (LCV detection) | Cy5 (CanCV detection) | HEX (PCR validation) | Interpretation     |
|----------------------|------------------------|-----------------------|----------------------|--------------------|
| +                    | +                      | +                     | +                    | HLVd, LCV, CanCV + |
| +                    | -                      | +                     | +                    | HLVd, CanCV +      |
| +                    | +                      | -                     | +                    | HLVd, LCV +        |
| +                    | -                      | -                     | +                    | HLVd +             |
| +                    | +                      | -                     | -                    | HLVd, LCV +        |
| +                    | -                      | -                     | -                    | HLVd +             |
| -                    | +                      | +                     | +                    | LCV, CanCV +       |
| -                    | -                      | +                     | +                    | CanCV +            |
| -                    | +                      | -                     | +                    | LCV +              |
| -                    | -                      | +                     | -                    | CanCV +            |
| -                    | +                      | -                     | -                    | LCV +              |
| -                    | -                      | -                     | +                    | Negative result    |
| -                    | -                      | -                     | -                    | Invalid PCR        |

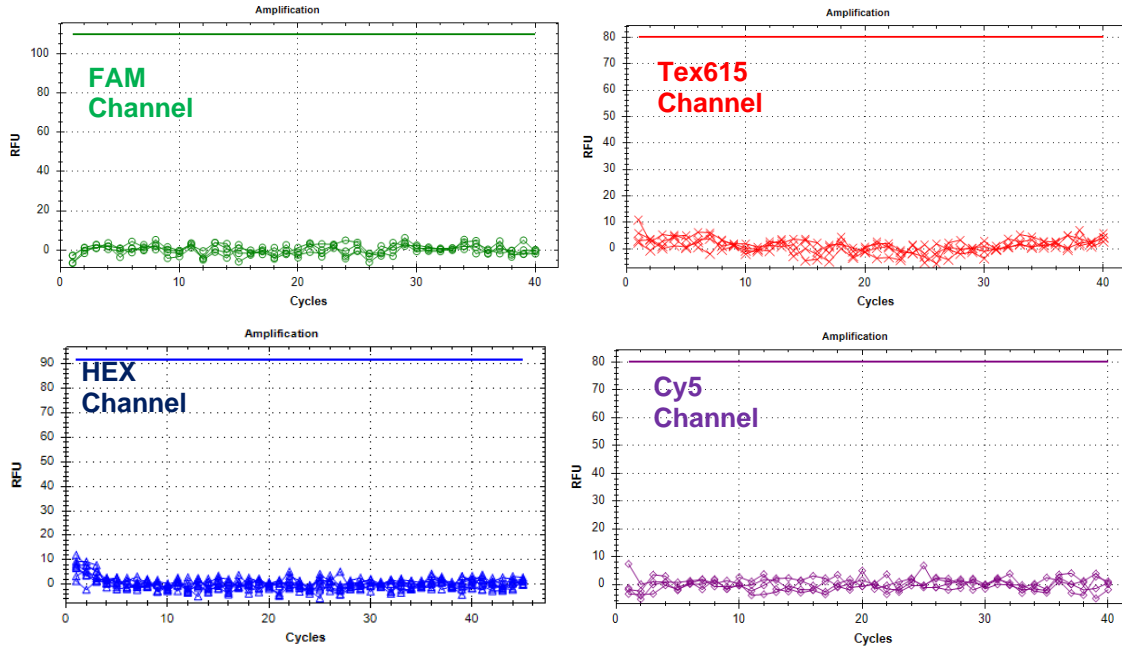
For results obtained that are not covered in Table 6, please refer to the Frequently Asked Questions.



**Figure 1.** Example of TaqMan One-step RT-PCR Positive result. PCR signals above the baseline from FAM, Tex615, Cy5, and HEX channel indicate the successful PCR. Individual amplification in any of the channels also indicates a positive result for the pathogen corresponding to that channel.



**Figure 2.** Example of TaqMan One-step RT-PCR Negative result. No target RNA was detected in FAM, TEX615, and Cy5 channels but amplification signal from HEX indicates the successful PCR.



**Figure 3.** Example of TaqMan one-step RT-PCR inhibition result. No signal from all targets was detected. It is suggested to repeat the sample preparation using recommended kit for RNA purification.

### E. HLVd/LCV/CanCV TaqMan RT-PCR Assay Specificity

The specificity of Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit is first and foremost ensured by the selection of the target-specific primers and probes, as well as the selection of stringent reaction conditions. The target primers were checked for possible homologies to all plant viroids in GenBank published sequences by sequence comparison analysis.

### Frequently Asked Questions

1. **How many samples should be included per RT-PCR run?**
  - This kit is designed to test 96 samples. For every 6 samples, a non-template control (Nuclease-Free Water) and a Positive Control must be included. It is preferable to collect and test 6 samples at a time.
  - To interpret the results correctly, the positive control should be amplifying at least the FAM, Cy5, and TEX615 channels, and the negative result be amplifying only the HEX channel.
2. **How should it be interpreted if no PCR control signal (HEX) is detected while one or more of the target specific signals (FAM, Cy5, Tex615) is detected in the sample?**
  - Tested samples(s) can be considered positive. The internal control is designed to be a weak signal to ensure it does not compete with the target and lower the sensitivity of the assay.
3. **How should it be interpreted if a target specific signal (FAM, Cy5, Tex615) and/or the PCR control signal (HEX) are detected in the negative control?**
  - It could happen when there is carryover contamination and PCR inhibition. Repeat the assay using fresh aliquots and clean pipette tips.
4. **How should it be interpreted if no target signal (FAM, Cy5, Tex615) is detected in positive control?**
  - It could happen when the positive control was not added. Repeat the assay.

| Related Products                               | Product #    |
|--|--------------|
| HLVd TaqMan Probe/Primer and Control Set       | TM38710      |
| Plant/Fungi RNA Purification Kit               | 25800, 31350 |
| Plant/Fungi Total RNA Purification 96-Well Kit | 31900        |

## Technical Support

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.

Technical support can also be obtained from our website ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

## Product Use Restriction

Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR.

Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

The presence of PCR inhibitors may cause false negative or invalid results.

The respective user is liable for any and all damages resulting from application of Norgen's HLVd/LCV/CanCV TaqMan RT-PCR Kit for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

TaqMan is a registered trademark of Roche Molecular Systems, Inc

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