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Coconut Cadang-Cadang Viroid (CCCVd) TaqMan RT-PCR Kit

Product# TM47950 **Product Insert**

Intended Use

Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) TaqMan RT-PCR Kit is designed for the detection of CCCVd specific RNA in a real-time PCR based on the use of TaqMan® technology. This kit is designed for research use only and not for use in diagnostic procedures.

Background Information

Cadang-cadang is a lethal disease of coconut (Cocos nucifera) caused by the *Coconut cadangcadang viroid* (CCCVd). The CCCVd is the smallest known pathogen and is biologically distinct from other viroids. The viroid consists of circular or linear single-stranded RNA with a size of 246 or 247 nucleotides. The viroid is mechanically transmissible via nucleic acid inocula, however the mode of natural transmission is not known. The viroid is only detected in Palmae. The mode of natural transmission is unknown and eradication measures fail to control the disease. Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) RT-PCR Detection Kit is a rapid and sensitive CCCVd detection tool which can be applicable for the early diagnosis of CCCVd.

Product Description

Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) TaqMan RT-PCR Kit is designed for the detection of CCCVd specific RNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures. The detection of CCCVd specific RNA is based on TaqMan one-step RT-PCR providing a simple, reliable and rapid result for the detection of CCCVd infection. CCCVd 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. The CCCVd TaqMan RT-PCR Kit comprises 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.

Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) TaqMan RT-PCR 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 # TM47950 (100 preps)
MDx TaqMan 2X RT-PCR Master Mix	2 x 0.7 mL
CCCVd Primer & Probe Mix	0.28 mL
CCCVd Positive Control	0.15 mL
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 and HEX filter channel
- 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 *Coconut Cadang-Cadang Viroid* (CCCVd) TaqMan RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) 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 the CCCVd genome 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 *Coconut Cadang-Cadang Viroid* (CCCVd) TaqMan RT-PCR Kit. The quality of the RNA template will have a major impact on the performance of the CCCVd 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 *Coconut Cadang-Cadang Viroid* 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 PCR Master Mix provided is enough for up to 128 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 CCCVd 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 CCCVd 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 µL
MDx TaqMan 2X RT-PCR Master Mix	10 µL
CCCVd Primer & Probe Mix	2 µL
Total Volume	20 µL

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

Table 2. TaqMan One-step RT-PCR CCCVd Assay Preparation

RT-PCR Components	Target detection (with MDx TaqMan 2x RT-PCR Master Mix)
Nuclease-Free Water	5 µL
MDx TaqMan 2X RT-PCR Master Mix	10 µL
CCCVd Primer & Probe Mix	2 µL
Sample RNA*	3 µL
Total Volume	20 µL

* The recommended amount of sample RNA to be used is 3 µL. However, a volume between 1 and 5 µL of sample RNA may be used as template. Adjust the final volume of the RT-PCR reaction to 20 µL 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 µL
CCCVd Primer & Probe Mix	2 µL
CCCVd Positive Control (PosC)	8 µL
Total Volume	20 µL

4. **OPTIONAL -** Experienced User Protocol for PCR Reaction Preparation for Multiple Samples

Follow Table 4 and the instructions below to prepare a PCR reaction mix for multiple samples. The below example is to set up 10 PCR reactions.

Table 4. Set-Up of PCR Reaction Mix for 10 PCR Reactions

Components	Volume per 10 + 1 Reactions
MDx TaqMan 2X RT-PCR Master Mix	110 µL
CCCVd Primer & Probe Mix	22 µL
Total Volume	132 µL

1. Aliquot 12 µL of the PCR reaction mix to PCR tubes or wells on a 96-well PCR plate.
2. For the negative control, add 8 µL of Nuclease free water and close the PCR tube cap.
3. For the detection samples, add 5 µL of water and 3 µL of template (DNA) to samples. Close the PCR tube cap.
4. For the positive control, add 8 µL of CCCVd Positive Control (PosC) and close the PCR tube cap.
5. Spin the tubes or 96-well plate briefly.
6. The final PCR reaction volume is 20 µL.

C. CCCVd TaqMan One-Step RT-PCR Assay Programming

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

Table 4. CCCVd TaqMan One-Step RT-PCR 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**	**95°C**	**15 sec**
	Step 2	**60°C**	**30 sec**

D. CCCVd TaqMan One-Step RT-PCR Assay Interpretation

Table 5. Interpretation of Assay Results

FAM (Target detection)	HEX (PCR validation)	Result
+	+	Positive
-	+	Negative
-	-	PCR inhibited

For results obtained that are not covered in Table 5, please refer to the Frequently Asked Questions.

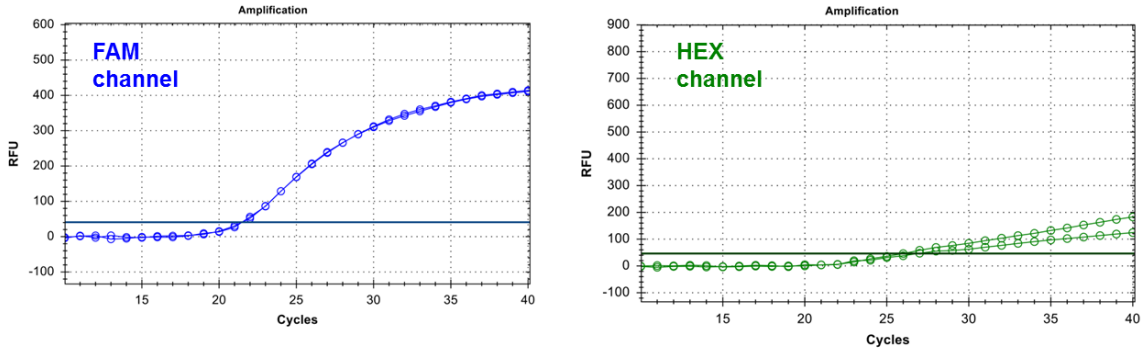

Figure 1. Example of TaqMan One-step RT-PCR Positive result. Both PCR signals above the baseline from FAM and HEX channel indicate the successful PCR.

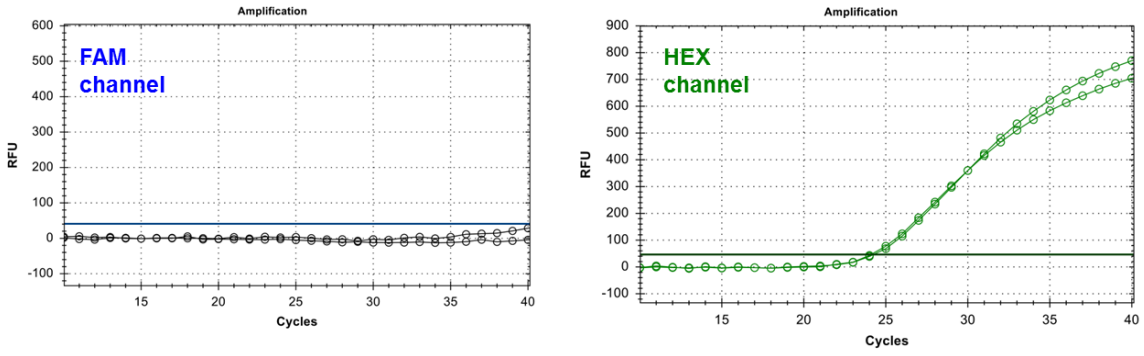

Figure 2. Example of TaqMan One-step RT-PCR Negative result. No target RNA was detected in FAM channel but amplification signal from HEX indicates the successful PCR.

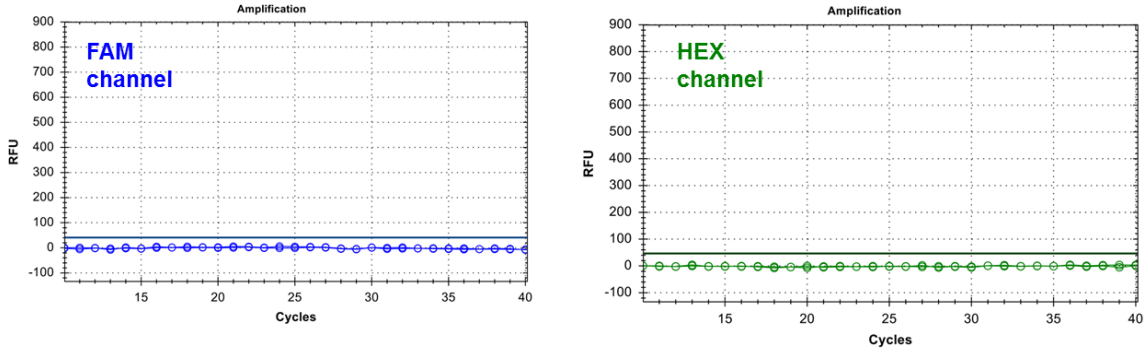

Figure 3. Example of TaqMan one-step RT-PCR inhibition result. No signal from both FAM and HEX channel was detected. It is suggested to repeat the sample preparation using recommended kit for RNA purification.

E. CCCVd TaqMan RT-PCR Assay Specificity

The specificity of Norgen's CCCVd RT-PCR Detection Kit is first and foremost ensured by the selection of the CCCVd-specific primers, as well as the selection of stringent reaction conditions. The CCCVd 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?**
   - Norgen's Coconut Cadang-Cadang Viroid (CCCVd) TaqMan RT-PCR Detection 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.

2. **How should it be interpreted if no PCR control signal (HEX) is detected while the target specific signal (FAM) is detected in the positive control?**
   - Tested samples(s) can be considered positive. It could happen when too much target RNA template was added due to the preferential amplification on the target.

3. **How should it be interpreted if the target specific signal (FAM) and/or the PCR control signal (HEX) are detected in the negative control?**
   - It could happen when there are 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) is detected in positive control?**
   - It could happen when the positive control was not added. Repeat the assay.

Related Products	Product #
CCCVd TaqMan Probe/Primer and Control Set	TM47910
Plant RNA/DNA Purification Kit	24400
Plant/Fungi RNA Purification Kit	25800
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) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) TaqMan RT-PCR Kit is designed for the detection of CCCVd specific RNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) 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.

Potential mutations within the target regions of the CCCVd genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

The respective user is liable for any and all damages resulting from application of Norgen's *Coconut Cadang-Cadang Viroid* (CCCVd) 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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