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Chrysanthemum Stunt Viroid (CSVd) End-Point RT-PCR Kit Product Insert

Product# EP39900

Intended Use

Norgen's *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR Kit is designed for the detection of CSVd specific viroid RNA based on the use of end-point RT-PCR technology. This kit is designed for research use only and not for use in diagnostic procedures.

Background Information

The *chrysanthemum stunt viroid* (CSVd) is the causal agent of a disease known as chrysanthemum stunt. The viroid consists of small pieces of RNA (354 bp) that are known to be highly contagious. The main symptoms of CSVd infection are severe stunting of the plants such that they are half to two-thirds of the normal size, as well as smaller flowers and premature flowering. Symptoms of Chrysanthemum Stunt are difficult to detect in stock, however can be so severe at flowering that finished crops of flowers are unmarketable. There is no chemical that can control CSVd, therefore much effort has been made to generate chrysanthemum plants free from CSVd. Norgen's Chrysanthemum Stunt Viroid RT-PCR Detection Kit is a rapid and sensitive CSVd detection tool which can be applicable for the early diagnosis of CSVd

Product Description

Norgen's *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR Kit is designed for the detection of CSVd specific RNA based on the use of end-point RT-PCR technology. This kit is designed for research use only and not for use in diagnostic procedures. The kit includes Master Mix and primers for the specific amplification of a 342 nucleotide region of the CSVd genome, as well as a positive control and a negative control to confirm the integrity of the kit reagents. In addition, the kit contains loading dye and a DNA ladder to facilitate analysis of the results.

The detection of CSVd specific RNA is based on end-point RT-PCR technology, utilizing Reverse transcription polymerase chain reaction (RT-PCR) for the amplification of specific CSVd RNA sequences. For analysis of the RT-PCR data, the RT-PCR reaction is loaded on an agarose RNA gel along with the provided DNA ladder for qualitative analysis.

Norgen's *Chrysanthemum Stunt Viroid* (CSVd) End-Point 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

Kit Components

Component	Product # EP39900 (24 preps)
MDx 2X RT-PCR Master Mix	350 µL
CSVd Primer Mix	70 µL
CSVd Positive Control	50 µL
Nuclease-Free Water	1.25 mL
Loading Dye	100 µL
DNA Ladder	100 µL
Product Insert	1

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 reagents can be stored for 1 year at -20°C without showing any reduction in performance.

Customer-Supplied Reagents and Equipment

- Appropriate End-point PCR Instrument
- 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
- Agarose gel electrophoresis apparatus
- UV transilluminator with suitable gel documentation system
- 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 *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Norgen's *Chrysanthemum Stunt Viroid* (CSVd) End-Point 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 CSVd End-Point RT-PCR Kit 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 *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR Kit. The quality of the RNA template will have a major impact on the performance of the CSVd detection test. The user must ensure that the method used for RNA purification is compatible with end-point 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 *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR Kit.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers is used, 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. RT-PCR Assay Preparation

Notes:

- Before use, suitable amounts of all 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 2X RT-PCR Master Mix provided is enough for up to 32 RT-PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For every RT-PCR run, one reaction containing CSVd 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 RT-PCR run is 6.
- To avoid any contamination while preparing the 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 CSVd 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) Master Mix; 3) Primer Mix; and 4) the Sample RNA or Positive Control).

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

Table 1. RT-PCR Negative Control Preparation

RT-PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	8 µL
MDx 2X RT-PCR Master Mix	10 µL
CSVd Primer Mix	2 µL
Total Volume	20 µL

2. Prepare the RT-PCR reaction for sample detection as shown in Table 2 below. The recommended amount of sample RNA to be used is 2.5 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.

Table 2. RT-PCR CSVd Assay Preparation

RT-PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	5.5 µL
MDx 2X RT-PCR Master Mix	10 µL
CSVd Primer Mix	2 µL
Sample RNA	2.5 µL
Total Volume	20 µL

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

Table 3. RT-PCR Positive Control Preparation

RT-PCR Components	Volume Per PCR Reaction
MDx 2X RT-PCR Master Mix	10 µL
CSVd Primer Mix	2 µL
CSVd Positive Control (PosC)	8 µL
Total Volume	20 µL

C. CSVd 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. *CSVd RT-PCR* 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	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 4	**Step 1**	**72°C**	**5 min**
Cycle 5	**Step 1**	**4°C**	**∞**

D. CSVd RT-PCR Assay Interpretation

- For the analysis of the RT-PCR data, the entire 20 µL RT-PCR reaction should be loaded on a 1X TAE 1.4 % Agarose DNA gel along with 10 µL of Norgen's DNA Ladder (provided).
- The RT-PCR products should be resolved on the 1X TAE, 1.4 % Agarose gel at 150V for 30 minutes (Gel running time will vary depending on an electrophoresis apparatus).

Table 5. Interpretation of Assay Results

Input Type	Target amplification		Interpretation
	CSVd Target Band (342 bp)	**PCR control Band (150 bp)**	
Positive Control	Yes	Yes	Valid
Positive Control	Yes	No	Valid
Negative Control	No	Yes	Valid
Sample	Yes	Yes	Positive
Sample	No	Yes	Negative
Sample	No	No	PCR inhibition
Sample	Yes	No	Positive

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

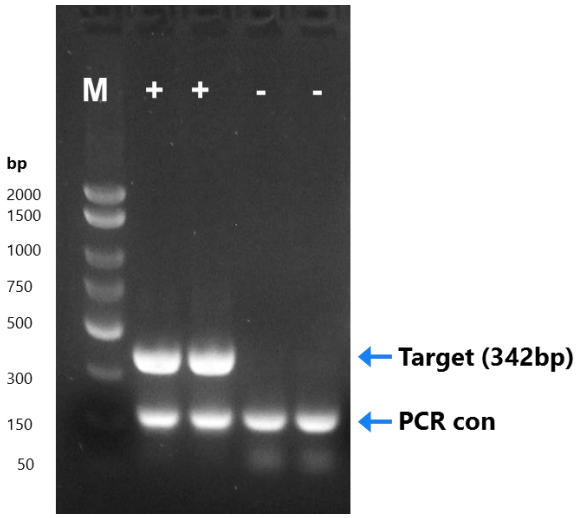

Figure 1: A representative 1X TAE, 1.4 % agarose gel showing the amplification of CSVd at different concentrations. The size of the CSVd target amplicon corresponds to the 342 bp band represented by the provided DNA Marker (M).

E. CSVd RT-PCR Assay Specificity

The specificity of Norgen's *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR Kit is first and foremost ensured by the selection of the CSVd-specific primers, as well as the selection of stringent reaction conditions. The CSVd 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 *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR 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 collect and test 6 samples at a time.

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

- The assay has to be repeated. It could happen when there is carryover contamination.

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

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

Related Products	Product #
Chrysanthemum Stunt Viroid (CSVd) SYBR Green RT-PCR Kit - 24 Reactions	SG39900
Chrysanthemum Stunt Viroid (CSVd) Primer and Control Set - 100 Reactions	EP39910
Chrysanthemum Stunt Viroid (CSVd) TaqMan RT-PCR Kit - 24 Reactions	TM39900
Chrysanthemum Stunt Viroid (CSVd) TaqMan Probe/Primer and Control Set - 100 Reactions	TM39910
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 *Chrysanthemum Stunt Viroid* (CSVd) End-Point RT-PCR Kit is designed for the detection of CSVd specific viroid RNA based on the use of end-point RT-PCR technology. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen's *Chrysanthemum Stunt Viroid* (CSVd) End-Point 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 CSVd 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 *Chrysanthemum Stunt Viroid* (CSVd) End-Point 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.

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