Information pest: *Potato spindle tuber viroid*

Potato spindle tuber viroid (PSTVd) is a regulated plant pathogen included in European legislation as a quarantine pest.

PSTVd is one of the most important diseases of potato and tomato. It is a small, unencapsidated, covalently closed, circular RNA of around 359 nucleotides.

PSTVd is easily transmissible by contact and hence may be mechanically disseminated from plant to plant or with contaminated equipment, tools, skin or clothes during field operations. Cutting seed potatoes before planting increase the risk of disseminating the viroids. Besides infected seed-tubers, PSTVd is also transmitted by the pollen of potato and tomato flowers.

PSTVd is a serious threat for host crops such as the potato and the tomato. In potato, PSTVd can induce severe growth reduction; however, reduction may also be hardly visible. Veins of infected plants may be smaller, more upright, and produce smaller leaves than their healthy counterparts. Infected tubers may be small, elongated (from which the disease derives its name), misshapen and cracked. The first symptoms of PSTVd infection in tomato are growth reduction and chlorosis in the top of the plant. Subsequently, this growth reduction may develop into stunting.

Introduction

The qPCR PSTVd set has been developed and optimized by Qualiplante
This product should be used only for research purposes.

Intended use

The qPCR PSTVd set is validated for the detection of *Potato spindle tuber viroid* (PSTVd) in One-Step Real-Time RT-PCR.

Suitable tissues are leaf tissue.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7PSTVdq2	qPCR *Potato spindle tuber viroid* (PSTVd) set 24
7PSTVdq9	qPCR *Potato spindle tuber viroid* (PSTVd) set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7PSTVdq2-DM-	2x48 tests 7PSTVdq9-DM-
RT-Enzyme	24 tests 7PSTVdq2-RT-	96 tests 7PSTVdq9-RT-
Positive Control	3 tests 7PSTVdq2-PC-	8 tests 7PSTVdq9-PC-
Negative Control	3 tests 7PSTVdq2-NC-	8 tests 7PSTVdq9-NC-

Storage conditions

This set can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the set is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

Materials and equipment (not provided)

- RNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- Thermal-cycler for Real-Time PCR with filters calibrated for FAM®

Nucleic acids extraction

Extract RNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

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Preparation of the PSTVd 1-Step master mix

a) Slowly thaw Direct Master Mix and RT-Enzyme by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and RT-Enzyme and spin down the liquid.
c) In a new tube called **PSTVd 1-Step master mix**, mix 14,5 µl of Direct Master Mix and 0,5 µl of RT-Enzyme per reaction. Do not forget to count the Positive Control and the Negative Control in the number of reactions to prepare.

Example:	**1 rxn**	**10 rxns**
Direct Master Mix	14,5 µl	145,0 µl
RT-enzyme	0,5 µl	5,0 µl

d) Store the **PSTVd 1-Step master mix** by placing it on ice or at 4°C.

Reaction set-up

a) Shake briefly **PSTVd 1-Step master mix** and spin down the liquid.
b) Add 15 µl of **PSTVd 1-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
c) Add 5 µl of RNA template to the **PSTVd 1-Step master mix**. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the Positive Control and the Negative Control.

Components	**Volume/PCR tube or well**
RNA template or Positive control or Negative control	5 µl
PSTVd 1-Step master mix	15 µl
Total Volume / PCR tube or well	20 µl

In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.

Run and thermal cycling

a) Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
b) Load the PCR tubes or plate into the thermal-cycler and follow the thermal cycling below:

Steps	**Temp (°C)**	**Time**	**Cycle(s)**
Reverse transcription	50°C	15 min	1
Enzyme activation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing and elongation	60°C	60 sec	

Results analysis

The reaction for PSTVd will generate a specific FAM®-labeled amplification curve.

Fig.1: Example of an amplification curve relative to a PSTVd positive sample.

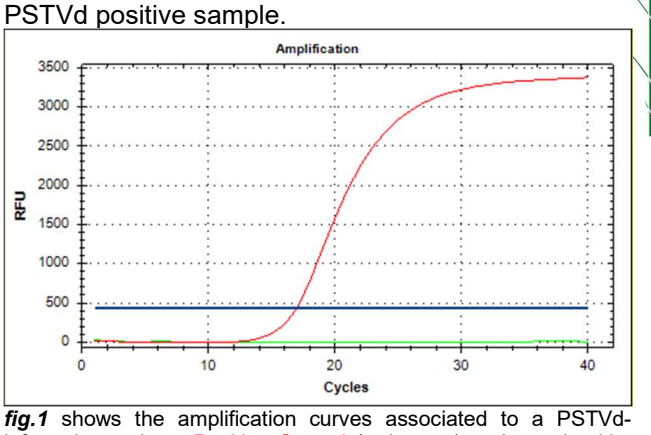

fig.1 shows the amplification curves associated to a PSTVd-infected sample or Positive Control (red curve) and to a healthy sample or Negative Control (green curve)

ANALYSIS VALIDATION

The PCR plate is validated only when:

- ✓ the Positive Control generates an amplification curve for FAM® fluorophore. The Cycle threshold (Ct) value of the FAM®-labeled amplification curve should be below or equal to 37 (***fig.1***).
- ✓ the Negative Control does not generate any amplification curve associated to FAM® fluorophore or a curve is generated for FAM® fluorophore but the Ct is higher than 40 (***fig.1***).

RESULTS INTERPRETATION

When all the previous conditions are performed, the amplification results are interpreted as indicated in the **tab. 1**, by considering for each sample the Ct of the curve generated by FAM® fluorochrome specific to PSTVd.

Ct	Interpretation
Ct ≤ 37	Positive
37 < Ct < 40	Indeterminate
Ct ≥ 40	Negative

tab.1 shows the results interpretation

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Special handling instructions

This set was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA/RNA and the components of the set. Do not touch any set components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the set components. Do not use reagents after their expiration date.

Troubleshooting

Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate	Repeat the test using the appropriate tools to seal correctly the plate
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the thermal cycler supplier
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In case of doubt, contact us
Abnormal amplification	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles

No amplification reaction is observed in the positive control well, while other samples are positive:

Possible causes	Corrective actions
The positive control provided with the set was not added into the reaction well	Repeat the test. If the problem persists, contact us

An amplification plot is observed in the negative control well:

Possible causes	Corrective actions
Contamination of the negative control or the Master Mix with target-positive nucleic acid	Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly

Warranty and Responsibilities

Qualiplante SAS guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Sets. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits Qualiplante SAS responsibility to the replacement of the product. No other warranties, of any kind, express or implied are provided by Qualiplante SAS.
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The Sets have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Sets. The user is personally responsible for data that she/he will obtain and/or she/he will supply to third parties using these Sets. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the set can be returned and the user can be refunded.
Sets components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change.

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