Information pest: *Citrus tristeza virus*

Citrus tristeza virus (CTV) is a regulated plant pathogen included in European legislation as a quarantine pest. This Closterovirus causes one of the most harmful diseases affecting *Citrus* and it is one of the most economically important pathogens of the crop.

CTV is transmitted by several aphid species in a semipersistent manner; *Toxoptera citricidus* is the most efficient vector.

There are different strains of the virus, each producing different symptoms (slow decline with small leaves, yellowing and leaf fall, twig dieback and small fruit; quick decline with wilt and die; stem pitting; seedling yellows with yellow leaves and dieback branches) on different *Citrus* cultivars and rootstocks. Long-distance spread can occur by the movement of CTV-infected citrus planting material, or by the movement of plant material infested with CTV-infected aphids.

Introduction

The qPCR *Citrus tristeza virus* set was developed in collaboration with the company International Plant Analysis and Diagnostics (www.ipadlab.eu).

Primers and probe were designed according to the sequence of the most conserved region of the CTV genome. The assay was evaluated on most than 400 CTV isolates from different areas (Spain, Italy, Chile, Egypt…). No cross-reactions were observed for other citrus virus and viroids: *Citrus psorosis virus, Citrus vein enation virus, Citrus variegation virus, Citrus exocortis viroid, Hop stunt viroid, Citrus bent leaf viroid, Citrus viroid III and Citrus viroid IV.*

This product should be used only for research purposes.

Intended use

The qPCR set is validated for the detection of *Citrus tristeza virus (CTV)* in One-Step Real-Time RT-PCR. Suitable tissues are plant tissues (shoots, mature fruits including peduncle and columella, leaves including petioles) and aphids.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7CTV--q2	qPCR *Citrus tristeza virus* set 24
7CTV--q9	qPCR *Citrus tristeza virus* set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7CTV--q2-DM-	2x48 tests 7CTV--q9-DM-
RT-Enzyme	24 tests 7CTV--q2-RT-	96 tests 7CTV--q9-RT-
Positive Control	3 tests 7CTV--q2-PC-	8 tests 7CTV--q9-PC-
Negative Control	3 tests 7CTV--q2-NC-	8 tests 7CTV--q9-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 CTV 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 **CTV 1-Step master mix**, mix 17,5 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	17,5 µl	175,0 µl
RT-enzyme	0,5 µl	5,0 µl

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

Reaction set-up

a) Shake briefly **CTV 1-Step master mix** and spin down the liquid.
b) Add 18 µl of **CTV 1-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
c) Add 2 µl of RNA template to the **CTV 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	2 µl
CTV 1-Step master mix	18 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	55°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 *Citrus tristeza virus* will generate a specific FAM®-labeled amplification curve.

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

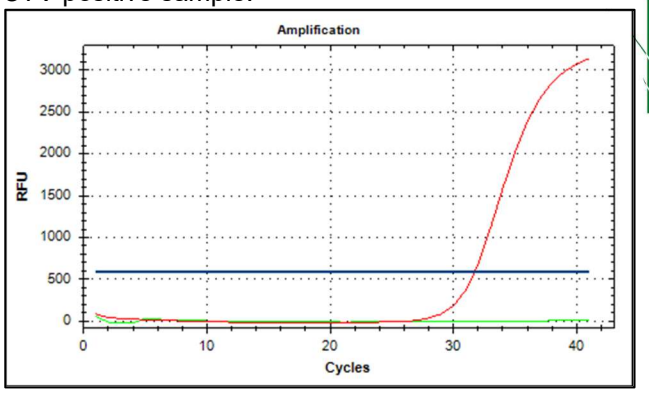

fig.1 shows the amplification curves associated to a *CTV*-infected sample or Positive Control (red curve) and to a healthy sample or Negative Control (green curve)

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 to 36 (***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 or equal than 36 (***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 *CTV*.

Ct	Interpretation
Ct < 36	Positive
Ct ≥ 36	Negative

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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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Sets components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change.

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