User Guide
PCR Citrus tristeza virus kit

Version 01 – 23/06/2021

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 PCR Citrus tristeza virus kit has been developed by Qualiplante based on Rubio et al. (2001). A verification was performed by Qualiplante (data not published) and the performance characteristics of the kit are the same as shown in the original publication. The primer pair amplifies a 561 bp product of genomic region P coding for a protein of unknown function that accumulates in amorphous inclusion bodies.
This product should be used only for research purposes.

Intended use

The PCR kit is validated for the detection of Citrus tristeza virus (CTV) in One-Step End-Point RT-PCR.

Suitable tissues are plant tissues (shoots, mature fruits including peduncle and columella, leaves including petioles) and aphids.

Kit format and content

Article N°	Product name
PCR CTV 96	PCR Citrus tristeza virus 96 tests

Content	96 tests
Direct Master Mix	2x48 tests
RT-Enzyme	96 tests
Positive Control	8 tests
Negative Control	8 tests

Storage conditions

This kit 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 kit 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
- DNA ladder and loading-dye buffer
- PCR thermal cycling
- Agarose gel reagents and apparatus

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	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	
Storage	4°C	∞	-

Agarose gel electrophoresis

Prepare an agarose gel **at 1,5% w/v in 1X-TAE buffer**.

Gel loading:
- load the DNA ladder (for example 100-1'000 bp DNA step ladder)
- load 10 µl of PCR products from the previous step adding the loading-dye buffer (not provided in the kit).

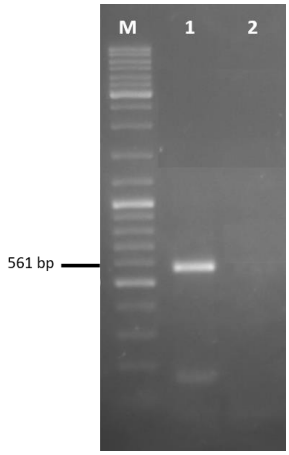
Run: run the gel electrophoresis for 50-60 minutes at 80V.

Results analysis

ANALYSIS VALIDATION

Citrus tristeza virus is detected when a 561 bp DNA fragment is observed. The analysis is validated when:
- ✓ 1 DNA fragment of 561 bp is visible in the positive control lane.
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 1X-TAE 1,5% agarose gel showing the DNA amplification in a sample infected by CTV:



MK: DNA ladder – **1: CTV positive sample** or Positive Control **- 2:** healthy sample or Negative Control

RESULTS INTERPRETATION

The specific product of CTV is a 561 bp DNA fragment.

- ✓ A sample is **positive** when a 561 bp specific DNA fragment is present in the PCR reaction.
- ✓ A sample is **negative** when no fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

Fragment size **561 bp**	Interpretation
-	Negative
✓	**POSITIVE** Citrus tristeza virus

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Special handling instructions

This kit 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 kit. Do not touch any kit 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 kits with different batch numbers in the same test procedure. Do not interchange reagents with other kits. 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 kit 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 kit 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

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