

## Information pest

Apple is one of the most widely grown fruit crops worldwide. Apple stem pitting virus (ASPV) is economically important and a common pathogen in commercial cultivars.

ASPV infections are latent in the great majority of commercial apple cultivars, whereas, in sensitive rootstocks, this virus elicits stem grooving, epinasty, that is, a marked curling of the leaves caused by necrosis of the main vein, and chlorotic/necrotic mottling of the leaves, followed by decline.

ASPV has no natural vector but is dispersed by human movement of infected plant.

## Introduction

The PCR Apple stem pitting virus kit has been developed by Qualiplate based on Menzel et al. (2002).

The primers pair was designed on highly conserved regions. The specificity of the primers pair was confirmed by sequencing the RT-PCR products.

*This product should be used only for research purposes.*

## Intended use

The PCR kit is validated for the detection of Apple stem pitting virus (ASPV) in One-Step End-Point RT-PCR.

Suitable tissues are leaves and bark chips containing vegetative buds.

## Kit format and content

Article N°	Product name
PCR ASPV 96	PCR Apple stem pitting virus 96 tests

Content	kit 96
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, Qualiplate can recommend you an extraction method.

## Preparation of the ASPV 1-Step master mix

- Slowly thaw **Direct Master Mix** and **RT-Enzyme** by placing it on ice or at 4°C.
- Shake briefly **Direct Master Mix** and **RT-Enzyme** and spin down the liquid.
- In a new tube called **ASPV 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

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

## Reaction set-up

- Shake briefly **ASPV 1-Step master mix** and spin down the liquid.
- Add 18 µl of **ASPV 1-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
- Add 2 µl of RNA template to the **ASPV 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 <b>Positive control</b> or <b>Negative control</b>	2 µl
<b>ASPV 1-Step master mix</b>	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

- 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.
- 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	30 sec	34
Annealing and elongation	55°C	60 sec	
Storage	4°C	∞	-

### Agarose gel electrophoresis

Prepare an agarose gel at **2% w/v** in **0,5X-TBE 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.

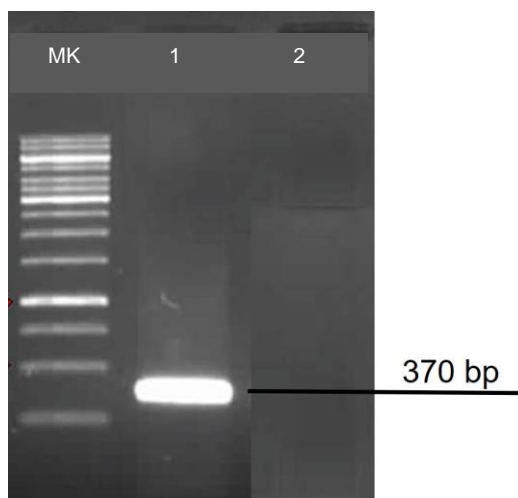
### Results analysis

#### ANALYSIS VALIDATION

Apple mosaic virus is detected when a 370 bp DNA fragment is observed. The analysis is validated when:

- ✓ 1 DNA fragment of 370 bp is visible in the positive control lane.
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 0,5X-TBE 2% agarose gel showing the RNA amplification in a sample infected by ASPV:



MK: DNA ladder - 1: ASPV positive sample or **Positive Control** of the kit - 2: healthy sample or **Negative Control** of the kit

#### RESULTS INTERPRETATION

The specific product ASPV is a 370 bp DNA fragment.

- ✓ A sample is **positive** when a 370 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 <b>370 bp</b>	Interpretation
-	Negative
✓	<b>POSITIVE</b> Apple stem pitting virus

### 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

Qualiplante SAS guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. 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 Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that she/he will obtain and/or she/he will supply to third parties using these Kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded.

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