

Information pest

Pseudomonas syringae pv. *actinidiae* (PSA) was included in European legislation as a quarantine pest.

PSA is the causal agent of the bacterial canker of *Actinidia* spp., the most damaging and severe disease of cultivated kiwifruits. The disease was first reported in Japan in 1989. From 2008 to date, severe epidemics of the bacterial canker were described in Central Italy, then in the rest of Europe.

PSA populations show genetic variability: five biovars are present in different world's areas, characterized by differences in virulence. Biovar 1 groups the strains associated with the initial epidemics of bacterial canker observed in Japan and Italy. Biovar 2 has only been isolated in South Korea. Biovar 3 corresponds to a highly virulent population. Biovar 4 was removed and classified in a new pathovar. Other strains are classified as biovars 5 and 6.

Symptoms of the disease are easily observed on aerial parts (trunks, leaders, canes, leaves, flowers and fruits). PSA causes brown discolouration of buds, dark brown angular spots surrounded by yellow haloes on leaves, cankers with white to reddish (oxydation) exudate on twigs and trunks, fruit collapse, wilting and eventually plant mortality. The most conspicuous symptom is the red-rusty exudation which covers bark tissues on trunks and twigs. Symptoms are usually expressed during spring and autumn when climatic conditions are favorable to the disease. The bacterium is spread by plants for planting *Actinidiae* spp.

Introduction

The SYBR *Pseudomonas syringae* pv. *actinidiae* kit has been developed by Qualiplante based on Gallelli et al.,2014. This PCR set is referred in the [PM7/120 \(2\) *Pseudomonas syringae* pv. *actinidiae*](#), European and Mediterranean Plant Protection Organization Bulletin (2021) 51, 549-567.

This kit is suitable for the detection of *P. syringae* pv. *actinidiae* biovar 3. The primer pair was designed in a region of the *hrpW* gene responsible of the pathogenicity of PSA and involved in the hypersensitive response.

Gallelli et al. (2014) and a TPS ([Eupresco PSADID Project](#)) that involved twelve laboratories permitted to collect data on the performance of the test:

- Analytical sensitivity: 10³ cfu mL⁻¹
- Analytical specificity: 100% inclusivity and exclusivity
- Repeatability: 94%
- Reproducibility: 91%
- Diagnostic sensitivity: 96,4%
- Diagnostic specificity: 90%
- Accuracy: 93,2%

This product should be used only for research purposes.

Intended use

The SYBR PSAg kit is validated for the detection of *Pseudomonas syringae* pv. *actinidiae* in Real-Time PCR (SYBR-Green® technology). The SYBR-Green® technology allows to confirm that a sample generating an amplification signal is produced only by nucleic acids of the pathogen of interest, by interpreting the melting peak.

Suitable tissues are plants, ooze drops, bleeding sap or pure culture suspension. leaves.

Kit format and content

Article N°	Product name
SYBR PSAg 96	SYBR PSAg 96 tests

Content	96 tests
Nuclease free water	2 x 1,2 ml
Primers mix	96 tests
Taq polymerase	2 x 0,2 ml
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)

- DNA 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 SYBR-Green®

Nucleic acids extraction

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

Reaction set-up

- Do a short spin in a centrifuge to collect the pellet at the bottom of the **Primers mix** tube.
- Add 1.400,00 µl (one thousand four hundred) of **nuclease free water** provided with the kit in the **Primers mix** tube. Vortex vigorously up and down the **Primers mix** tube. Spin down the liquid.
- Slowly thaw **Taq polymerase** by placing it on ice or at 4°C.
- Shake briefly **Taq polymerase** and spin down the liquid.
- For each reaction, in a new tube (not provided), prepare the **Master mix** by mixing 4 µL of **Taq polymerase** in 14 µl of **Primers mix**. Do not forget the **Positive Control** and **Negative Control** of the kit which must be added to check that the reaction is working correctly.

Example: if you have 8 samples to test, you will need 10 reactions (8 samples + 1 **Positive Control** + 1 **Negative Control**) of 18 µl each. In a tube, please, mix 40 µL of **Taq polymerase** in 140 µl of **Primers mix**.

- Add 18 µl of the freshly prepared **Master mix** (14 µl of **Primers mix** + 4 µl of **Taq polymerase**) to each PCR tubes or wells of an optical-grade PCR plate.
- Add 2 µl of DNA template. 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
DNA template or Positive control or Negative control	2 µl
Master mix (14 µl of Primers mix + 4 µl of Taq polymerase)	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)
Initial activation	95°C	12 min	1
Denaturation	95°C	15 sec	40
Annealing	72°C	20 sec	
Extension	72°C	20 sec	
Melt temperature	Follow the instructions of your thermal cycler		

Results analysis

The reaction for PSA will generate a specific SYBR®-labeled amplification curve and a specific melting-curve.

Fig.1: Example of amplification curves relative to a PSA positive sample and negative sample.

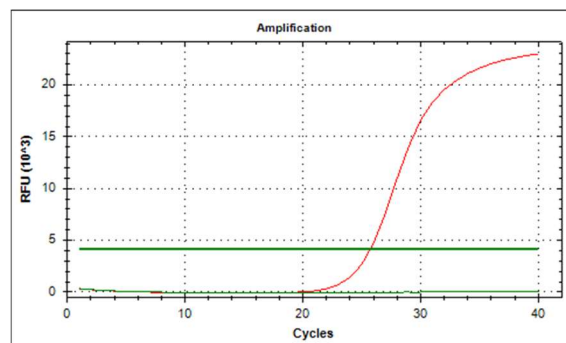


fig.1 shows the amplification curves associated to a PSA infected sample or **Positive Control** of the kit (red curve) and to a healthy sample or **Negative Control** of the kit (green curve).

Fig.2: Example of melting curves relative to a PSA positive sample and negative sample

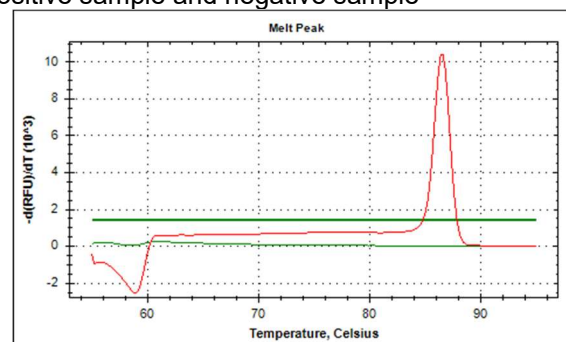


fig.2 shows the melting curve associated to a PSA infected sample or **Positive Control** of the kit (red curve - Tm=87,00°C) and to a healthy sample or **Negative Control** of the kit (green curve) on Biorad CFX96 machine.

ANALYSIS VALIDATION AND RESULTS INTERPRETATION

Both amplification curve and melting curve need to be analyzed.

For a correct interpretation of results, always:

- check if result of **Positive Control** generates an amplification curve with a Ct below 35 and a melting temperature (Tm) specific to the researched target.
- check if result of **Negative Control** does not generate any amplification curve, or if an amplification curve appears, that there is not any melting curve. If an amplification curve is observed, ensure that no melting curve is present and in the case that a melting curve is present, check that its Tm differs from the Tm of the researched target

If all the above conditions are observed, the results can be interpreted as indicated in **table 1** below considering the Ct the amplification curve generated by the SYBR-Green® specific to PSA and the melting curve.

Table 1: Interpretation of the results

Melting temperature	Ct Value	
	Ct < 40	No Ct
Tm differs no more than $\pm 1^\circ\text{C}$ from Tm of Positive Control	Positive	Repeat the analysis
Tm differs more than $\pm 1^\circ\text{C}$ from Tm of Positive Control	Negative	Negative

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 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:

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.

Qualiplante SAS is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting of the use and/or the misuses of the Kits. The user consciously and under her/his own responsibilities decides for the utilization purposes of the Kits and uses it the way she/he considers most suitable in order to reach her/his goals and/or objectives. Qualiplante SAS is not responsible for the data resulting from the use of the Kits, for the utilization that the user independently decides to make of them or for the direct or indirect damages possibly resulting from the disclosure or transmission of the data themselves to third parties under any form or circumstance. This clause is automatically accepted by the user when purchasing the Kits.

Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Qualiplante SAS does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. The product, equipment and information included in the Kits consist of assembled reagents.

The Kits are designed for the services supply, quality control or any other application that is not exclusively an internal company's research and requires a specific license for PCR and Real-Time PCR use. The license and authorization for PCR and Real-Time PCR use are not included in the Kits. The user is responsible for setting prefixed goals, choosing whether or not to perform the PCR or Real-Time PCR reaction and to apply for register her/his own license.

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.

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

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 any 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