

Information pest: *Pseudomonas syringae* pv. *actinidiae*

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.

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* set has been developed by QualiPlante based on Rees-George et al., 2010. This PCR set is referred in the PM7/120 (1) *Pseudomonas syringae* pv. *Actinidiae*, European and Mediterranean Plant Protection Organization Bulletin (2014) 44 (3), 360-375.

The primer pair was designed to be complementary to a portion of the 16S-23S rDNA intertranscribed spacer (ITS) region.

No cross reactions were observed from other bacterial species as *Agrobacterium rhizogenes*, *Clavibacter michiganensis* subsp. *michiganensis*, *Erwinia amylovora*, *Klebsiella pneumoniae*, *Erwinia carotovora* subsp. *carotovora*, *Erwinia chrysantemi*, *Pantoea agglomerans*, *Escherichia coli*, *Pseudomonas cichorii*, *Pseudomonas corrugata*, *Pseudomonas fluorescens*, *Pseudomonas marginalis*, *Pseudomonas syringae* pv. *papulans*, *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas viridiflava*, *Xanthomonas campestris* pv. *phaseoli*, *Xanthomonas campestris* pv. *populi*, *Xanthomonas campestris* pv. *pruri*, *Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas* sp.

Cross-reactions were observed from *Pseudomonas syringae* pv. *theae*, *Pseudomonas avellanae* and *Pseudomonas syringae* pv. *tomato*, but these bacteria are not present on *Actinidiae* plant.

This product should be used only for research purposes.

Intended use

The SYBR PCR set 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.

This set offers a sensitive diagnostic method to detect PSA and permits to avoid amplicon contamination in routine diagnosis. Suitable tissues from symptomatic plant can include trunk and leader parts, canes and shoots, including those showing cankers or other lesions, leaves, buds, flowers or fruits with necrotic spots or necrotic lesions.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7PSA--S2	SYBR <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> (PSA) - set 24
7PSA--S9	SYBR <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> (PSA) - set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7PSA--S2-DM-	2x48 tests 7PSA--S9-DM-
Positive Control	3 tests 7PSA--S2-PC-	8 tests 7PSA--S9-PC-
Negative Control	3 tests 7PSA--S2-NC-	8 tests 7PSA--S9-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)

- 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

- Slowly thaw **Direct Master Mix** by placing it on ice or at 4°C.
- Shake briefly **Direct Master Mix** and spin down the liquid.
- Add 18 µl of **Direct Master Mix** (without DNA template) to each PCR tubes or wells of an optical-grade PCR plate.
- Add 2 µl of DNA template to the **Direct 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
DNA template or Positive control or Negative control	2 µl
Direct 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

- 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 denaturation	95°C	12 min	1
Denaturation	95°C	15 sec	40
Annealing	65°C	30 sec	
Elongation	72°C	30 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 at different concentrations.

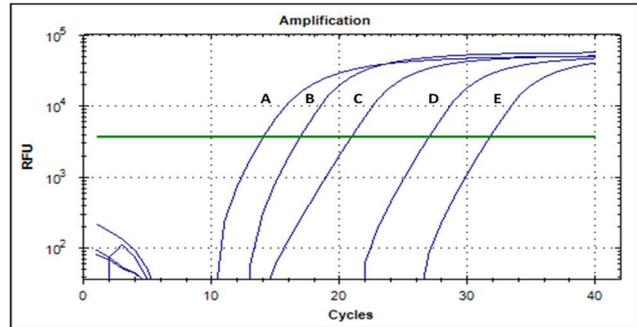


fig.1 shows the amplification curves associated to a PSA infected sample at different concentrations: **A:** 100 ng of gDNA - **B:** 10 ng of gDNA - **C:** 1 ng of gDNA - **D:** 0,1 ng of gDNA - **E:** 0,01 ng of gDNA

Fig.2: Example of melting curves relative to a PSA positive sample at different concentrations.

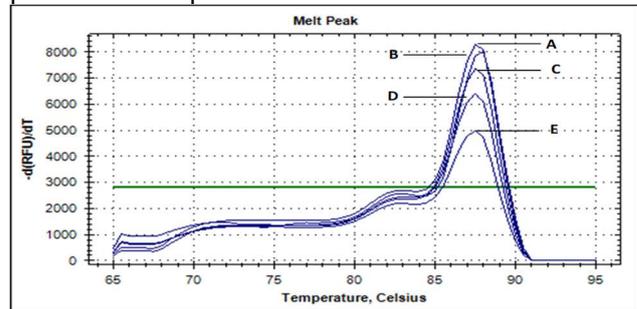


fig.2 shows the melting curve associated to a PSA infected sample (Tm=88,0°C) at different concentrations: **A:** 100 ng of gDNA - **B:** 10 ng of gDNA - **C:** 1 ng of gDNA - **D:** 0,1 ng of gDNA - **E:** 0,01 ng of gDNA, on Biorad CFX96 machine.

ANALYSIS VALIDATION AND RESULTS INTERPRETATION

For a correct interpretation of results, always:

- check if results of **Positive Control** and **Negative Control** pass,
- combine amplification curves analysis with melting curves analysis,
- confirm that the samples melting temperatures match the **Positive Control** melting temperature.

Step 1: Check the Ct values of Positive Control and Negative Control

Well	Ct	Interpretation
Positive Control	30 or less	Go to step 2 and 3
	Above 30 or no Ct	Fail*
Negative Control	No Ct	Go to step 2 and 3
	Less than 30	Fail**

* Repeat the assay, ensuring that steps of the user guide are carefully performed

** The mix or the **Negative Control** was contaminated with PSA nucleic acids. Repeat the assays after identifying and removing the potential source of contamination

Step 2: Check Ct value in the samples well

Well	Ct	Interpretation
Sample	35 or less	Go to step 3
	More than 35 or no Ct	Negative

Step 3: See melting temperature

Well	Tm	Interpretation
Sample	Tm differs no more than $\pm 1^\circ\text{C}$ from Tm of Positive Control	Positive
	Tm differs more than $\pm 1^\circ\text{C}$ from Tm of Positive Control	Negative

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 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 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

Qualiplate 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 Qualiplate SAS responsibility to the replacement of the product. No other warranties, of any kind, express or implied-are provided by Qualiplate SAS. Qualiplate 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 Sets. The user consciously and under her/his own responsibilities decides for the utilization purposes of the Sets and uses it the way she/he considers most suitable in order to reach her/his goals and/or objectives. Qualiplate SAS is not responsible for the data resulting from the use of the Sets, 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 Sets. 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. Qualiplate SAS does not encourage the unlicensed use of patented applications. The Sets 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 Sets consist of assembled reagents. The Sets 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 Sets. 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 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.