User Guide
SYBR PSAg kit
Version 01 – 15/09/2022

qualiplante

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 an 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 (Euphresco PSADID Project) that involved twelve laboratories permitted to collect data on the performance of the test:

- Analytical sensitivity: 10^3 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 interpretating 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
Direct Master Mix	2x48 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)

- 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.

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Reaction set-up

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

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)
UDG activation	50°C	2 min	1
Activation	95°C	2 min	1
Denaturation	95°C	15 sec	40
Annealing/Elongation	60°C	1 min	
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.

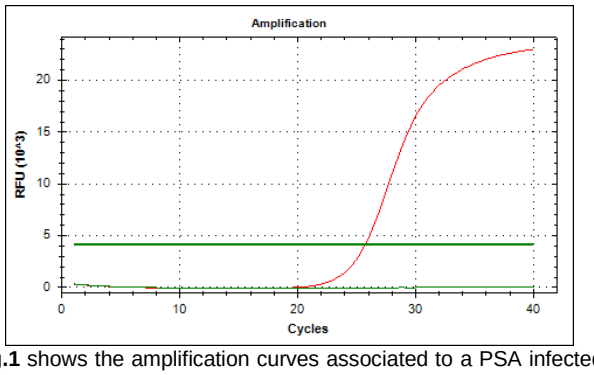

fig.1 shows the amplification curves associated to a PSA infected sample or Positive Control of the kit (red curve) and to a healthy sample or Negative Control of the kit (green curve).

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

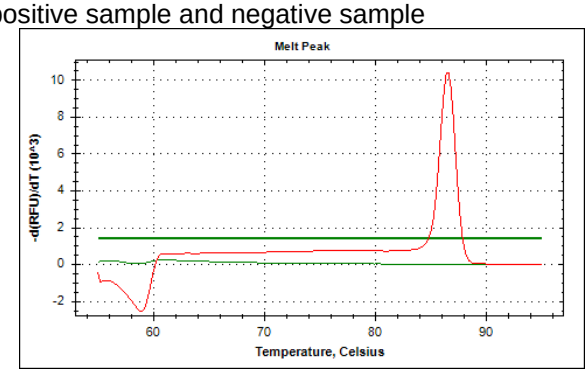

fig.2 shows the melting curve associated to a PSA infected sample or Positive Control of the kit (red curve - Tm=87,00°C) and to a healthy sample or Negative Control of the kit (green curve) on Biorad CFX96 machine.

ANALYSIS VALIDATION AND RESULTS INTERPRETATION

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	35 or less	Go to step 2 and 3
	Above 35 or no Ct	Fail*
Negative Control	No Ct	Go to step 2 and 3
	Less than 35	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

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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 ± 1°C from Tm of Positive Control	Positive
	Tm differs more than ± 1°C from Tm of Positive Control	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/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 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

Warranty and Responsibilities

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