

Information pest

Erwinia amylovora is a bacterium that causes fireblight, a devastating disease in particular for pome fruit trees. Fire blight is probably the most serious disease affecting *Pyrus* spp. (pear) and *Malus* spp. (apple) cultivars in many countries.

It received its name from the appearance of the infected leaves and branches which often appears blackened as if scorched by fire. Symptoms of fire blight on the principal hosts are relatively similar and easily recognized. Confusion between fire blight and blight- or blast-like symptoms, especially in blossoms and shoots, may occur with diseases/disorders caused by other bacteria, fungi, insect damage and physiological disorders, and consequently laboratory analysis is always necessary.

Erwinia amylovora is a regulated plant pathogen included in European legislation as a quarantine pest.

Introduction

The qPCR Ea has been developed by Qualiplante based on Pirc and al., 2009. A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication.

The primers and probe were designed on the *ams* gene, involved in the synthesis of the capsular polysaccharide amylovoran which is unique to *E. amylovora* and strongly associated with its multiplication in plants and its pathogenicity.

Validation data of the method are available from a test performance study realized in 2010 (Euphresco [ERWINDECT](#) project). This method is also referred in the Appendix 12 of the [PM7/20 \(2\)](#) *Erwinia amylovora*, European and Mediterranean Plant Protection Organization Bulletin (2013) 43 (1), 21-45.

This product should be used only for research purposes.

Intended use

The qPCR set is validated for the detection of *Erwinia amylovora* in Real-Time PCR. The test can be applied to any kind of plant material or bacterial colonies.

Symptomatic samples can be processed individually or in small batches combining material from several samples, taking precaution during the DNA extraction step to avoid cross-contamination. Samples should preferably be composed of flowers, shoots or twigs, leaves, fruitlets (all with necrosis and with exudates if possible), or the discoloured subcortical tissues (after peeling bark from cankers in twigs, branches, trunk or collar).

The detection of *E. amylovora* in **asymptomatic plants** has been shown to be difficult. The analyses of asymptomatic plants should be performed in

summer or early autumn to increase the likelihood of detecting the bacteria.

Sampling and sample preparation are described in the Appendix 1 of the PM7/20 (2) from EPPO.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7Ea---q2	qPCR Ea set 24
7Ea---q9	qPCR Ea set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7Ea---q2-DM-	2x48 tests 7Ea---q9-DM-
Positive Control	3 tests 7Ea---q2-PC-	8 tests 7Ea---q9-PC-
Negative Control	3 tests 7Ea---q2-NC-	8 tests 7Ea---q9-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 FAM®.

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)
Enzyme activation	95°C	12 min	1
Denaturation	95°C	15 sec	40
Annealing and elongation	60°C	60 sec	

Results analysis

The reaction for *Erwinia amylovora* will generate a specific FAM®-labeled amplification curve.

Fig.1: Example of an amplification curve relative to a *Erwinia amylovora* positive sample.

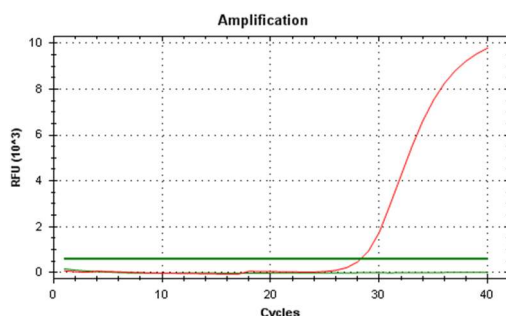


fig.1 shows the amplification curve associated to a **Ea-infected sample** or **Positive control** (red curve) and the amplification curve associated to a healthy sample or **Negative control** (green curve).

ANALYSIS VALIDATION

The PCR plate is validated only when:

- ✓ the **Positive Control** generates an amplification curve for FAM® fluorophore. The Cycle threshold (Ct) value of the FAM®-labeled amplification curve should be below to 38.
- ✓ the **Negative Control** does not generate any amplification curve associated to FAM® fluorophore or a curve is generated for FAM® fluorophore but the Ct is higher or equal to 38.

RESULTS INTERPRETATION

When all the previous conditions are performed, the amplification results are interpreted as indicated in the **tab. 1**, by considering for each sample the Ct of the curve generated by FAM® fluorochrome specific to *Erwinia amylovora*.

Ct	Interpretation
Ct < 38	Positive
38 ≤ Ct < 40	Indeterminate(*)
Ct ≥ 40	Negative

(*) The test should be repeated.

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 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

Qualiplante 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 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 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. Qualiplante 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. Qualiplante 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.