Information pest

Ralstonia solanacearum was originally described by Smith (1896) as the causative agent of bacterial wilt infected more than 450 plant species from 50 botanical families, including important crops such as potato, tomato, eggplant, pepper, tobacco and banana. This soil bacterium is the causal agent of a severe and devastating disease of major economic importance on *Solanaceous* crops.

Ralstonia solanacearum has been subclassified into three distinct species: *R. solanacearum* (Phylotype II, referenced as of South American origin), *Ralstonia pseudosolanacearum* (Phylotype I, referenced as of Asian origin and III as of African highlands) and *Ralstonia syzygii* (Phylotype IV, referenced as of Indonesian origin). One genotype assigned to Phylotype IIB sequevar 1, formerly referred to as race 3 biovar 2 and known as the causal agent of potato brown rot, is of particular importance to the EPPO region, having spread from South America with the movement of infected seed potato around the world.

Ralstonia solanacearum is a regulated plant pathogen included in European legislation as a quarantine pest.

Introduction

The qPCR Rsol set has been developed by Qualiplante based on Weller and al., 2000. A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication.

The broad-host-range R. solanacearum probe (RS-P) is partially homologous to 16S rRNA gene primer OLI1 (24), with primers (RS-I and RS-II) flanking this region. This set permits the detection of all strains of *R. solanacearum* and *R. pseudosolanacearum* and the specific detection and identification of *R. solanacearum* Phylotype II sequevar 1 (race 3) only. The broad-range probe (RS-P) detected all biovars of *R. Solanacearum*.

In pure culture, detection of *Ralstonia solanacearum* to ≥ 10^{-2} cells ml^{-1} was achieved; sensitivity decreased when the assay was performed with inoculated potato tissue extracts, prepared by currently recommended extraction procedures.

No cross reactions were observed with *Ralstonia pickettii*, *Ralstonia* sp., *Burkholderia andropogonis*, *Burkholderia caryophylli, Burkholderia cepacia, Burkholderia glumae, Burkholderia plantarii, Bacilus polymyxa, Pseudomonas marginalis* subsp. *marginalis, Pseudomonas chlorophis, Enterobacteriaceae, Ratinella aquatillis, Ochrobactrum anthropic,* but cross reactions were observed with Banana blood disease bacterium, *Ralstonia syzygii* strains, *Pseudomonas* sp. (Taxom B).

This method is also referred in the Appendix 5 of the PM 7/21 (2) *Ralstonia solanacearum, R. pseudosolanacearum* and *R. syzygii* (*Ralstonia solanacearum* species complex), European and Mediterranean Plant Protection Organization Bulletin (2018) 48 (1), 32-63.

This product should be used only for research purposes.

Intended use

The qPCR set is validated for the detection of *Ralstonia solanacearum* in Real-Time PCR. The test can be applied to bacterial colonies isolated from potato tuber tissue or directly in potato tuber tissue.

Sampling and sample preparation are described in the PM7/21 (2) from EPPO.

Set format and content

Two sets are available for 24 and 96 tests.

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

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

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Nucleic acids extraction

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

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)
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 *Ralstonia solanacearum* will generate a specific FAM®-labeled amplification curve.

Fig.1: Example of an amplification curve relative to a *Ralstonia solanacearum* positive sample.

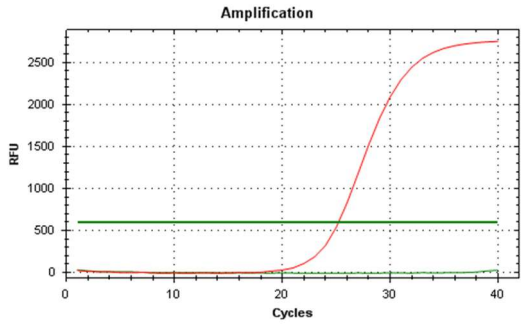

fig.1 shows the amplification curve associated to a Rsol-infected sample or Positive control (red curve) and the amplification curve associated to a healthy sample or Negative control (green curve).

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 or equal to 35.
- ✓ 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 to 35.

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 *Ralstonia solanacearum*.

Ct	Interpretation
Ct ≤ 35	Positive
No Ct or Ct > 35	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 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.

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

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