## Information pest: *Grapevine leafroll-associated virus 1*

Grapevine is subject to a number of important graft-transmissible diseases. *Grapevine leafroll-associated virus 1* (GLRaV-1) is a grapevine's virus classified in the *Ampelovirus* genus and in the *Closteroviridae* family. It is a causal agent of the leafroll disease. Infected plant and most of their part (such as leaves, shoots, canes, trunks and root system) are smaller than healthy plants. In late summer, leaves roll downwards, and the interveinal area of the leaf blade becomes bright yellow or red. Fruit ripening is delayed.

GLRaV-1 is a regulated non-quarantine pest in Europe.

## Introduction

The qPCR *Grapevine leafroll-associated virus 1* set has been developed by Qualiplante based on Osman et al., 2007. A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication.
The *Grapevine leafroll-associated virus 1* set offers a sensitive diagnostic method to detect the presence within the plant of the virus. The performance of the method has been demonstrated using several nucleic acids extraction procedures and a wide range of geographically distributed isolates (South Africa, Europe, Australia, Asia, Latin America and United States). The hHSP70 (homologous heat-shock 70 protein) gene was used to design primers and probes.
*This product should be used only for research purposes.*

## Intended use

The qPCR set is validated for the detection of *Grapevine leafroll-associated virus 1* (GLRaV-1) in One-Step Real-Time RT-PCR. Suitable tissues are grapevine leaves and bark scrapings from dormant canes.

## Set format and content

Two sets are available for 24 and 96 tests.

| Article N° | Product name |
|---|---|
| 7GLRa1q2 | qPCR GLRaV-1 set 24 |
| 7GLRa1q9 | qPCR GLRaV-1 set 96 |

| Content | set 24 | set 96 |
|---|---|---|
| Direct Master Mix | 24 tests 7GLRa1q2-DM- | 2x48 tests 7GLRa1q9-DM- |
| RT-Enzyme | 24 tests 7GLRa1q2-RT- | 96 tests 7GLRa1q9-RT- |
| Positive Control | 3 tests 7GLRa1q2-PC- | 8 tests 7GLRa1q9-PC- |
| Negative Control | 3 tests 7GLRa1q2-NC- | 8 tests 7GLRa1q9-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)

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


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## Preparation of the GLRaV-1 One-Step master mix

a) Slowly thaw Direct Master Mix and RT-Enzyme by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and RT-Enzyme and spin down the liquid.
c) In a new tube called **GLRaV-1 One-Step master mix**, mix 17,5 µl of Direct Master Mix and 0,5 µl of RT-Enzyme per reaction. Do not forget to count the Positive Control and the Negative Control in the number of reactions to prepare.

| Example: | 1 rxn | 10 rxns |
|---|---|---|
| Direct Master Mix | 17,5 µl | 175,0 µl |
| RT-enzyme | 0,5 µl | 5,0 µl |

d) Store the **GLRaV-1 One-Step master mix** by placing it on ice or at 4°C.

## Reaction set-up

a) Shake briefly **GLRaV-1 One-Step master mix** and spin down the liquid.
b) Add 18 µl of **GLRaV-1 One-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
c) Add 2 µl of RNA template to the **GLRaV-1 One-Step 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 |
|---|---|
| RNA template or Positive control or Negative control | 2 µl |
| **GLRaV-1 One-Step 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) |
|---|---|---|---|
| Reverse transcription | 50°C | 15 min | 1 |
| Enzyme activation | 95°C | 10 min | 1 |
| Denaturation | 95°C | 15 sec | 40 |
| Annealing and elongation | 60°C | 60 sec | |

## Results analysis

The reaction for *Grapevine leafroll-associated virus 1* will generate a specific FAM®-labeled amplification curve.

**Fig.1:** Example of an amplification curve relative to a GLRaV-1 positive sample.

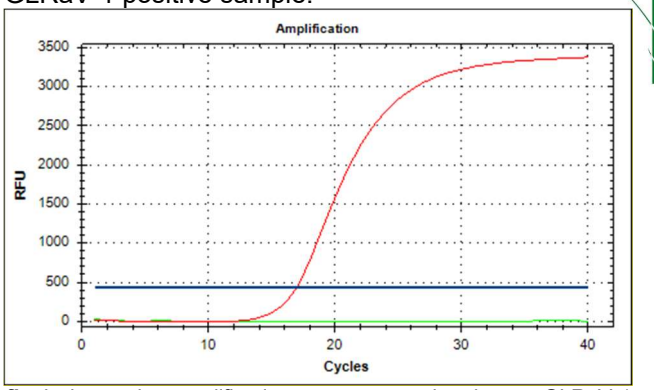


***fig.1*** shows the amplification curves associated to a GLRaV-1 infected sample or Positive Control (red curve) and to a healthy sample or Negative Control (green curve)

### 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 38 (***fig.1***).
- ✓ 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 40 (***fig.1***).

### 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 GLRaV-1.

| Ct | Interpretation |
|---|---|
| Ct ≤ 38 | Positive |
| 38 < Ct < 40 | Uninterpretable (*) |
| No Ct or Ct ≥ 40 | Negative |

***tab.1*** shows the results interpretation
(*) Test should be repeated


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## 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 case of 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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