

Information pest: *Grapevine leafroll-associated virus 3*

Grapevine is subject to a number of important graft-transmissible diseases. *Grapevine leafroll-associated virus 3* (GLRaV-3) is a grapevine's virus classified in the *Ampelovirus* genus and in the *Closteroviridae* family. It is the most important causative agent involved in grapevine 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-3 can be transmitted by grafting and by mealybugs and/or soft scale insects.

GLRaV-3 is a regulated non-quarantine pest in Europe.

Introduction

The SYBR GLRaV-3 set has been developed by Qualiplante and adapted from Osman and Rowhani, 2006.

The *HSP70-like protein* gene was used to design primers and probes.

This product should be used only for research purposes.

Intended use

The SYBR PCR set is validated for the detection of *Grapevine leafroll-associated virus 3* by 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.

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	
7GLRa3S2	SYBR GLRaV-3 set 24	
7GLRa3S9	SYBR GLRaV-3 set 96	

Content	set 24	set 96
Direct Master Mix	24 tests 7GLRa3S2-DM-	2x48 tests 7GLRa3S9-DM-
Positive Control	3 tests 7GLRa3S2-PC-	8 tests 7GLRa3S9-PC-
Negative Control	3 tests 7GLRa3S2-NC-	8 tests 7GLRa3S9-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 SYBR-Green®

Nucleic acids extraction

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

Reverse transcribe the RNA extracted from your samples into complementary DNA (cDNA) according to your usual protocol.

Reaction set-up

- a) Slowly thaw **Direct Master Mix** by placing it on ice or store at +4°C.
- b) Shake briefly **Direct Master Mix** and spin down the liquid.
- c) Add 18 µl of **Direct Master Mix** (without cDNA template) to each PCR tubes or wells of an optical-grade PCR plate.
- d) Add 2 µl of cDNA 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
cDNA 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)
UDG activation	50°C	2 min	1
Enzyme 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 GLRaV-3 will generate a specific SYBR®-labeled amplification curve and a specific melting-curve.

Fig.1: Example of amplification curves.

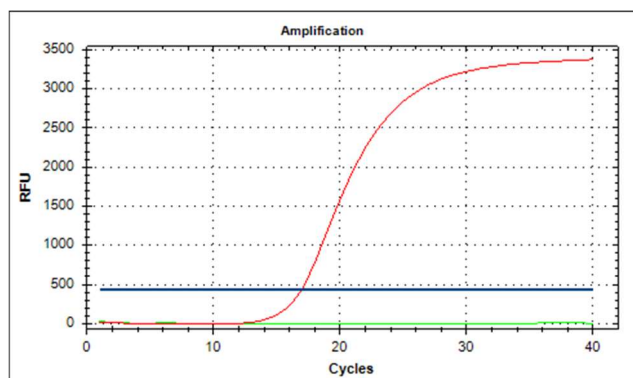


fig.1 shows the amplification curves associated to a GLRaV-3 infected sample or **Positive Control** (red curve) and to a healthy sample or **Negative Control** (green curve)

Fig.2: Example of melting curves relative to GLRaV-3 positive sample.

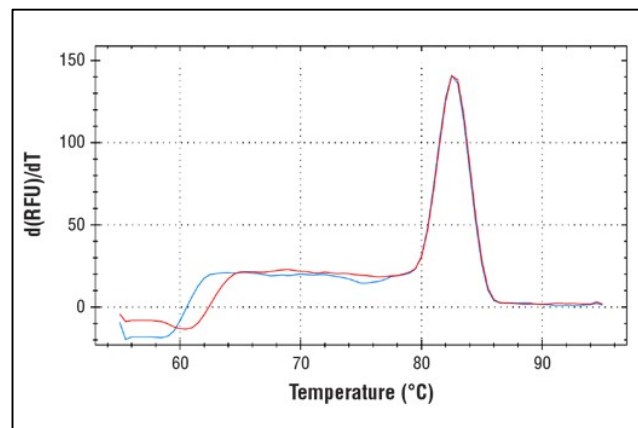


fig.2 shows melting curves associated to GLRaV-3 infected samples or **Positive control** of the set ($T_m=83,0^{\circ}\text{C}$ on Biorad CFX96 machine).

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	38 or less	Go to step 2 and 3
	Above 38 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 GLRaV-1 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	38 or less	Go to step 3
	More than 38 or no Ct	Negative

Step 3: See melting temperature

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

Possible causes	Corrective actions
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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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.

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