

Information pest: *Grapevine virus A*

Grapevine is subject to a number of important graft-transmissible diseases. *Grapevine virus A* (GVA) is a grapevine's virus classified in the *Vitivirus* genus and in the *Betaflexiviridae* family. It is associated with the rugose wood complex, reported from almost all grape growing regions of the world, and mainly characterized by stem pitting and stem growing. GVA is frequently found in association with other viruses. The expression of the disease is influenced by other viruses including *Grapevine leafroll-associated viruses*.

Because GVA has been reported to occur in most major grapevine-producing countries, it poses no phytosanitary risk. However, it is recommended that only certified virus-free stocks be exchanged in national and international trade.

Introduction

The qPCR *Grapevine virus A* set has been developed by Qualiplante based on Margaria et al. 2009 and Osman and Rowhani, 2008.

The *Grapevine virus A* set offers a sensitive diagnostic method to detect the presence within the plant of the virus.

The CP (Coat 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 virus A* (GVA) in One-Step Real-Time RT-PCR. Suitable tissues are grapevine leaves collected outside hot periods (for example in September/October) and bark scrapings from dormant canes.

Set format and content

Two sets are available for 24 and 96 tests.

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

Content	set 24	set 96
Direct Master Mix	24 tests 7GVA--q2-DM-	2x48 tests 7GVA--q9-DM-
RT-Enzyme	24 tests 7GVA--q2-RT-	96 tests 7GVA--q9-RT-
Positive Control	3 tests 7GVA--q2-PC-	8 tests 7GVA--q9-PC-
Negative Control	3 tests 7GVA--q2-NC-	8 tests 7GVA--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)

- 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.

Preparation of the GVA One-Step master mix

- Slowly thaw **Direct Master Mix** and **RT-Enzyme** by placing it on ice or at 4°C.
- Shake briefly **Direct Master Mix** and **RT-Enzyme** and spin down the liquid.
- In a new tube called **GVA 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

- Store the **GVA One-Step master mix** by placing it on ice or at 4°C.

Reaction set-up

- Shake briefly **GVA One-Step master mix** and spin down the liquid.
- Add 18 µl of **GVA One-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
- Add 2 µl of RNA template to the **GVA 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
GVA 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

- 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)
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 virus A* will generate a specific FAM[®]-labeled amplification curve.

Fig.1: Example of an amplification curve relative to a GVA positive sample.

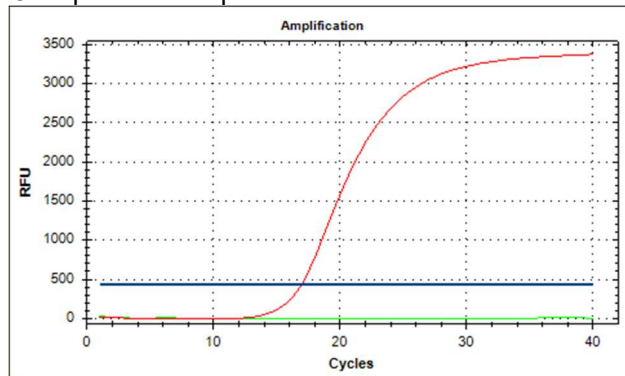


fig.1 shows the amplification curves associated to a GVA infected sample or **Positive Control** (red curve) and to a healthy sample or **Negative Control** (green curve)

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 GVA.

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

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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