

Information pest: *Grapevine fleck virus*

Grapevine fleck virus (GFkV) is a grapevine's virus classified in the *Maculavirus* genus and in the *Tymoviridae* family. GFkV infects grapevines worldwide.

GFkV is a graft-transmissible virus that causes obvious symptoms of fleck disease only in *Vitis rupestris*. Other *Vitis* species can be infected but remain symptomless. Symptoms include clearing of the veinlets in young leaves, which can spread into a mosaic pattern in older leaves. Older leaves may also become distorted and curl upwards. Symptoms are observed in spring during mild weather and disappear with the onset of hotter temperatures.

GFkV is a virus only found in the phloem and is transmitted through propagation and grafting. Field spread of GFkV has been reported in few countries although no vector is known.

GFkV is a regulated non-quarantine pest in Europe.

Introduction

The qPCR *Grapevine fleck virus* 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 fleck virus* 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 RdRp (RNA-dependent RNA polymerase) 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 fleck virus* (GFkV) 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
7GFkV-q2	qPCR GFkV set 24
7GFkV-q9	qPCR GFkV set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7GFkV-q2-DM-	2x48 tests 7GFkV-q9-DM-
RT-Enzyme	24 tests 7GFkV-q2-RT-	96 tests 7GFkV-q9-RT-
Positive Control	3 tests 7GFkV-q2-PC-	8 tests 7GFkV-q9-PC-
Negative Control	3 tests 7GFkV-q2-NC-	8 tests 7GFkV-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 GFkV 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 **GFkV 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 **GFkV One-Step master mix** by placing it on ice or at 4°C.

Reaction set-up

- Shake briefly **GFkV One-Step master mix** and spin down the liquid.
- Add 18 µl of **GFkV 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 **GFkV 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
GFkV 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 fleck virus* will generate a specific FAM®-labeled amplification curve.

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

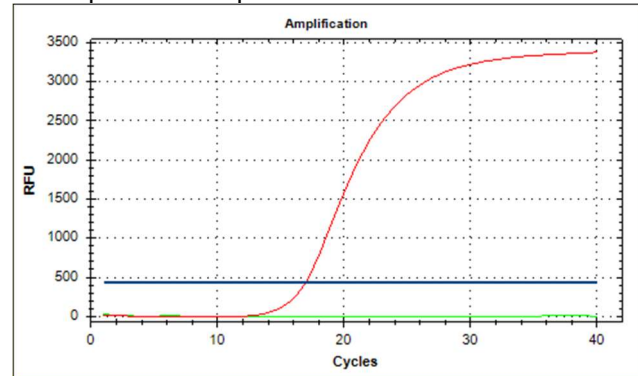


fig.1 shows the amplification curves associated to a GFkV infected sample or **Positive Control** (red curve) and to a healthy sample or **Negative Control** (green curve)

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 GFkV.

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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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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