User Guide
PCR Grapevine Pinot gris virus kit

Version 02 – 22/07/2021

qualiplante

Information pest: Grapevine Pinot gris virus

Grapevine Pinot gris virus (GPGV) is a member of the genus *Trichovirus* in the family *Betaflexiviridae*. This virus was recently identified in the north of Italy, in 2012, in the variety Pinot gris. Since then, it has been reported in numerous different countries and has been confirmed in other wine and table grape varieties including Pinot noir, Traminer, Chardonnay, Merlot, Cabernet Franc, Cabernet Sauvignon, Carménère, Glera, Sauvignon Blanc and Shiraz.

Grapevines infected with GPGV can either show symptoms (delayed budburst, lead distortion and mottling, shortened shoot internodes, poor yield…) or can be asymptomatic. Symptoms appear most distinct at the start of the season and are less apparent on late season growth, in both young and old vineyards.

GPGV is a graft-transmitted virus and is possibly transmitted by grape leaf bud and blister mites.

Introduction

The PCR Grapevine Pinot gris virus kit has been developed by Qualiplante.

The motor gene (*MP* gene) was used to design the primer pair.
This product should be used only for research purposes.

Intended use

The PCR GPGV kit is validated for the detection of Grapevine Pinot gris virus in One-Step End-Point RT-PCR.

Suitable tissues are grapevine young leaves and bark scrapings from dormant canes.

Kit format and content

Article N°	Product name
PCR GPGV 96	PCR Grapevine Pinot gris virus 96 tests

Content	96 tests
Direct Master Mix	2x48 tests
Positive Control	8 tests
Negative Control	8 tests

Storage conditions

This kit 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 kit 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
- DNA ladder and loading-dye buffer
- PCR thermal cycler
- Agarose gel reagents and apparatus

Nucleic acids extraction

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

Preparation of the GPGV 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 **GPGV 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 **GPGV One-Step master mix** by placing it on ice or at 4°C.

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Reaction set-up

a) Slowly thaw **GPGV One-Step master mix** by placing it on ice or at 4°C.
b) Shake briefly **GPGV One-Step master mix** and spin down the liquid.
c) Add 18 µl of **GPGV One-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
d) Add 2 µl of RNA template to the **GPGV One-Step master mix**. Do not forget to prepare a PCR tube or well of a 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
GPGV 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 PCR thermal cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Reverse transcription	50°C	15 min	1
Initial denaturation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing/Elongation	56°C	60 sec	
Storage	4°C	∞	-

Agarose gel electrophoresis

Prepare an agarose gel **at 1,5% w/v in 1X-TAE buffer.**

Gel loading:
- load the DNA ladder (for example 100-1'000 bp DNA step ladder).
- load 10 µl of PCR products from the previous step adding the loading dye buffer (*not provided in the kit)*.

Run: run the gel electrophoresis for 50-60 minutes at 80V.

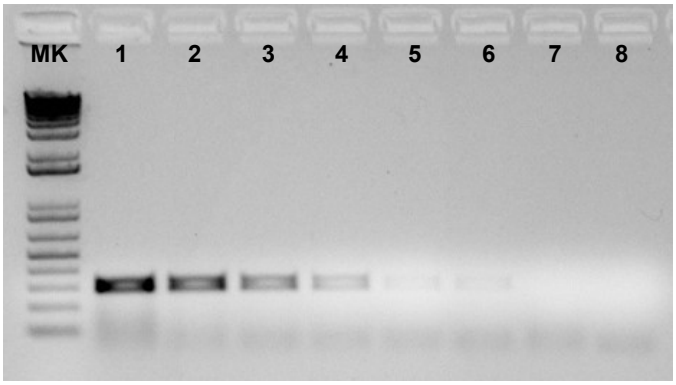
Results analysis

ANALYSIS VALIDATION

The specific product of Grapevine Pinot gris virus is a **302 bp fragment**.

The analysis is validated when:
- ✓ 1 DNA fragment of 302 bp is visible in the positive control lane.
- ✓ none DNA fragment is visible in the negative control lane.

The picture below represents a 1X-TAE 1,5% agarose gel showing the DNA amplification in a sample infected by Grapevine Pinot gris virus at different concentrations:



MK: DNA ladder - Sample infected by GPGV at different concentrations (or Positive control of the kit): **1:** 124,6 ng of total RNA - **2**: 12,46 ng of total RNA - **3**: 2,49 ng of total RNA - **4:** 1,25 ng of total RNA - **5:** 0,25 ng of total RNA - **6:** 0,12 ng of total RNA - **7:** Healthy sample or Negative control of the kit - **8:** No template control.

RESULTS INTERPRETATION

- ✓ A sample is infected by Grapevine Pinot gris virus if a 302 bp DNA specific fragment is present in the PCR reaction.
- ✓ A sample is not infected by Grapevine Pinot gris virus if none DNA fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

Fragment size **302 bp**	Interpretation
-	NEGATIVE
✓	**POSITIVE** GPGV

POSITIVE: infected sample – NEGATIVE: healthy sample

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Special handling instructions

This kit 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 kit. Do not touch any kit 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 kits with different batch numbers in the same test procedure. Do not interchange reagents with other kits. 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 kit 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 correctly seal 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, please, 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 kit was not added into the reaction well	Repeat the test. If the problem persists, please, 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

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