BIOREBA

qPCR grapevine red blotch virus (GRBV) set

User Guide

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1. Introduction

1.1 General

The qPCR GRBV set enables the simultaneous detection (multiplex) of grapevine red blotch virus (GRBV) and a *Vitis vinifera* host gene control (IC) with real-time PCR. Viral DNA, extracted from grapevine samples, is amplified in a qPCR reaction. The amplification of the DNA can be monitored in real-time because the specific probes are labeled with fluorophores (GRBV: FAM; IC: Cy5).
Suitable tissue sources of viral DNA are grapevine leaves (virus titer is generally higher in petioles from mature leaves) or wood scrapings (phloem). BIOREBA recommends to homogenize samples using the BIOREBA homogenizer system HOMEX and BIOREBA extraction bags "Universal" (Art.No 430100), and to follow a CTAB buffer-based method for DNA extraction.
The qPCR for the detection of GRBV was developed in collaboration with Agroscope, the Swiss center of excellence for research in the agriculture and food sector.

1.2 Special handling instructions

Perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing nucleic acid and the components of the set. Do not touch any set components with an ungloved hand. Keep all components tightly sealed when not in use. Use appropriate laboratory disposable parts. In particular, use nuclease-free tubes and filter tips to avoid degradation and cross-contamination.
Do not use components from sets with different lot numbers in the same test procedure. In order 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.

1.3 Warranty and liability

BIOREBA products are guaranteed to meet the specifications described on the product certificate of analysis and in the user guide, which is included with every shipment. No further warranties are given. If you have any questions about specifications or performance, please contact our administrative office (admin@bioreba.ch).

Our products are for laboratory use only and are not intended for human or animal applications. Should a product fail for reasons other than inappropriate handling or misuse, BIOREBA AG will replace the product free of charge or refund the purchase price.

BIOREBA AG shall not be liable for any direct or indirect, special or consequential damage of any kind resulting from the use of our products.

2. Intended use

The qPCR GRBV set is validated for the simultaneous detection (multiplex) of grapevine red blotch virus (GRBV) and internal control (IC) in real-time PCR. Suitable tissues are grapevine leaves or wood scrapings (phloem) (detection possible throughout the year).

3. Format, content, storage conditions and quality

3.1 Set format and contents

		Components		
Sales Part No.	Product name	Colour of screw cap	Name	Volume
879600	qPCR GRBV set 96	Green	Taq Master Mix (2x) Art. No. 830412	1.2 ml
		Clear	Primers/Probes Mix_GRBV/IC (10x) Art. No. 870100	0.2 ml
		–	Nuclease-free water Art. No. T143.4	1 ml
		–	GRBV DNA positive control (PC) Art. No. 870053	30 µl
		–	Plant DNA negative control (NC) Art. No. 870043	30 µl
879200	qPCR GRBV set 192	Green	Taq Master Mix (2x) Art. No. 830412	2x 1.2 ml
		Clear	Primers/Probes Mix_GRBV/IC (10x) Art. No. 870100	2x 0.2 ml
		–	Nuclease-free water Art. No. T143.4	2x 1 ml
		–	GRBV DNA positive control (PC) Art. No. 870053	30 µl
		–	Plant DNA negative control (NC) Art. No. 870043	30 µl

3.2 Storage conditions

Store all qPCR components (Taq Master Mix (2x), Primers/Probes Mix_GRBV/IC (10x), Nuclease free Water) at -30° C to -10° C. Avoid freeze/thaw cycles.

3.3 Lot-to-Lot consistency

Quality control of the qPCR set is performed based on pre-determined specifications to ensure consistent product quality. See lot-dependent certificate of analysis included with the shipment.

4. Materials and equipment (not provided)

- Nuclease-free filter tips and micropipettes
- Optical grade Nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- Thermocycler for real-time PCR
- Extraction bags "Universal" and HOMEX

5. Protocol

Please pay attention to the following points:

- The protocol in this manual must be followed.
- Create a nuclease-free environment by cleaning the bench with 1 % bleach followed by 70 % ethanol.
- Gloves must be worn at all times.
- Use nuclease-free tubes and filter tips.
- Use appropriate eye protection and wear protective clothing.
- To avoid cross-contamination, use separate rooms for
  a) nucleic acid extraction,
  b) preparation of the Master Mix and
  c) amplification.
- Avoid unnecessary freeze-thaw cycles of the qPCR components.

5.1 DNA extraction

For the extraction of DNA it is recommended to use standard DNA extraction protocols established for plant material. Upon request, BIOREBA can recommend an optimal extraction method.

5.2 Preparation of the qPCR samples

1. Slowly thaw the set components on ice or at 4 °C. Thereafter the components should always be kept on ice.

2. Shake the tubes briefly, and spin down the liquid.

3. To prepare the reaction mix, first determine the number of reactions and then increase the number by 1 or 2.

4. Prepare the reaction mix (without DNA template) by combining the components of the set to reach a final volume of 20 µl per reaction (see Table 1).

Component	Volume
Taq Master Mix (2x) Green	10 µl
Primers/Probes Mix_GRBV/IC (10x) Clear	2 µl
DNA Template	2 – 5 µl
Nuclease-free water	to reach a final reaction volume of 20 µl

Table 1: Preparation of reaction mix

5. Add the reaction mix (without DNA template) to each PCR tube or well of an optical-grade PCR plate.

6. Add 2 – 5 µl DNA template to the reaction mix.

7. Seal the PCR tubes or PCR plates, centrifuge briefly to collect components at the bottom of the PCR tubes or wells. Protect from light before thermocycling.

5.3 Thermal cycling

Place the PCR tubes or PCR plate in a thermocycler. Start cycling according to the program below (Table 2).

Step	Cycles	Temperature	Time
Hot start	1	95 °C	2 min
Denaturation Annealing/Extension	40	95 °C 60 °C	15 sec 30 sec

Table 2: PCR cycling conditions

5.4 Monitoring the PCR amplification

To monitor the simultaneous PCR amplification an appropriate thermocycler is required, which can measure the fluorescence of the following fluorophores:

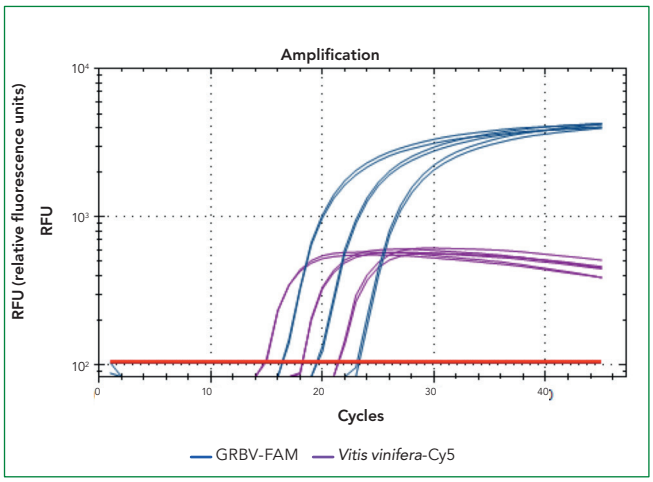
Dye	Virus/Control	Max. EX (nm)	Max. EX (nm)
FAM	GRBV	494	515
Cyanine 5	Vitis host gene (IC)	651	674

Table 3: Fluorophores overview

Please refer to the manufacturer's manual for information on programming the thermocycler, monitoring and evaluation.

5.5 Amplification of typical samples

Dilutions serie of a GRBV-positive sample (leaves from Gamay cultivar) showing amplification of GRBV (FAM, blue curves) and a host gene from *Vitis vinifera* (Cy5, purple curves).



Evaluation criteria
In order to distinguish positive from negative samples we recommend taking the following criteria into account:

A) The Ct value
B) The PCR efficiency
C) The delta RFU
(the difference between baseline and final RFU)

The range of values for each of the above can be determined for every channel by means of a dilution series of a known sample.

Version: 2 - 13.02.2023 - Changes on the format and adding information about the positive and negative control

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