BIOREBA

qPCR potato virus A (PVA) / potato virus M (PVM) set/kit

User Guide

Table of contents

1. Introduction

1.1 General

The qPCR set enables the simultaneous detection (multiplex) of potato virus A (PVA) and potato virus M (PVM) with real-time PCR. Viral RNA, extracted from potato samples, is amplified in a one-step RT-PCR reaction. The amplification of the cDNA can be monitored in real time because the specific probes are labeled with fluorophores (PVA: ROX and PVM: FAM). An internal control (IC: JOE) is included for the convenience of the operator. The control shows whether the reverse transcription and the amplification of the RNA worked as intended.
Suitable tissue sources of viral RNA are potato leaves and tubers (peel, stolon, sprouts). It is highly recommended to use the BIOREBA "Potato DNA/RNA rapid extraction" (included in the qPCR kits) for the extraction of RNA. The extraction method with "Potato DNA/RNA rapid extraction" has been validated and accredited according to ISO/IEC 17025. The method has been approved for the certification of seed potato.
BIOREBA recommends to homogenize samples using the BIOREBA homogenizer system HOMEX and BIOREBA extraction bags "Universal" (Art.No 430100).
The qPCR for the detection of PVA, PVM and IC was developed in collaboration with Agroscope, the Swiss centre of excellence for research in the agriculture and food sector.

Two sets and four kits are available for 96 or 192 extractions (see pages 4 and 5 for details).

1.2 Special handling instructions

Perform the tests in an RNase-free work environment. Always wear gloves when handling samples containing RNA and the components of the set. Do not touch any set/kit 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 set is validated for the simultaneous detection (multiplex) of potato virus A (PVA), potato virus M (PVM) and internal control (IC) in real- time PCR. Suitable tissues are potato leaves and tuber samples (peel, stolon and sprouts). Samples of up to 25 dormant tubers can be pooled for RNA extraction and analyzed according to this qPCR protocol.

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**
849600	qPCR PVA/PVM set 96	Blue	Taq Master Mix (2x) Art. No. 831412	1.2 ml
		Yellow	RT Master Mix (50x) Art. No. 830414	0.05 ml
		Clear	Primers/Probes/IC Mix_PVA/PVM/IC (10x) Art. No. 840100	0.2 ml
		–	Nuclease-free water Art. No. T143.4	1 ml
		–	PVA and PVM RNA positive control (PC) Art. No. 840053	30 µl
		–	Plant RNA negative control (NC) Art. No. 830043	30 µl
849200	qPCR PVA/PVM set 192	Blue	Taq Master Mix (2x) Art. No. 831412	2 x 1.2 ml
		Yellow	RT Master Mix (50x) Art. No. 830414	2 x 0.05 ml
		Clear	Primers/Probes/IC Mix_PVA/PVM/IC (10x) Art. No. 840100	2 x 0.2 ml
		–	Nuclease-free water Art. No. T143.4	2 x 1 ml
		–	PVA and PVM RNA positive control (PC) Art. No. 840053	30 µl
		–	Plant RNA negative control (NC) Art. No. 830043	30 µl

3.2 Kit format and contents

Part No.	**Descripton**	**No of reactions**	**Max pool size (potatoes)**
849610	**qPCR PVA/PVM kit 96/10** Includes: • qPCR PVA/PVM set 96 • Potato DNA/RNA rapid extraction set 96/10x for 96 extractions (Pool size: up to 10 tuber samples)	96	10
849625	**qPCR PVA/PVM kit 96/25** Includes: • qPCR PVA/PVM set 96 • Potato DNA/RNA rapid extraction set 96/25x for 96 extractions (Pool size: up to 25 tuber samples)	96	25
849210	**qPCR PVA/PVM kit 192/10** Includes: • qPCR PVA/PVM set 192 • Potato DNA/RNA rapid extraction set 192/10x for 192 extractions (Pool size: up to 10 tuber samples)	192	10
849225	**qPCR PVA/PVM kit 192/25** Includes: • qPCR PVA/PVM set 192 • Potato DNA/RNA rapid extraction set 192/25x for 192 extractions (Pool size: up to 25 tuber samples)	192	25

3.3 Storage conditions

Store all qPCR components (Taq Master Mix, RT Master Mix, Primers/Probes/IC Mix_PVA/PVM/IC, Nuclease free Water) at -30° C to -10° C. The components for rapid extraction (EB1, EB2) are stored at room temperature.

3.4 Lot-to-Lot consistency

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

4. Materials and equipment (not provided)

- RNase-free filter tips and micropipettes
- Optical grade RNase-free tubes/plate
- Disposable latex or vinyl gloves
- Thermal cycler for real-time PCR

5. Protocol

Please pay attention to the following points:

- The protocol in this manual must be followed.
- Create an RNase-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 / RNA extraction

The following protocol refers to BIOREBA`s Potato DNA/RNA rapid extraction set. If another DNA/RNA extraction method than the Potato DNA/RNA rapid extraction is used, please refer to the manufacturer`s user guide.

Put 0.2 g potato tissue into a clean grinding bag

Gently shake the rapid EB 1* and for single tests pipette** 500 µl rapid EB 1 into the grinding bag

Homogenize the potato tissue with a grinder

Transfer 100 µl homogenate avoiding the debris into a clean tube

Incubate the tube at 99.9 °C for 2 min then at 85 °C for 13 min and place immediately on ice

Spin the samples for 30 sec at 6000 g

Transfer 10 µl of supernatant without disturbing the pellet into a fresh tube containing 190 µl rapid EB 2

Vortex the sample briefly

Proceed with subsequent methods***

*Before shaking the buffer can show two phases
**For pools of 10 tubers use 1ml and for pools of up to 25 tubers use 2ml EB1
***For optimal results proceed with subsequent methods immediately

BIOREBA recommends to homogenize samples using the BIOREBA homogenizer system HOMEX and BIOREBA extraction bags. For more information please visit www.bioreba.ch or contact us by email or phone.

5.2 Preparation of the qPCR samples

1. Slowly thaw the kit components (with the exception of the RT Master Mix!) on ice or at 4°C. Thereafter the components should always be kept on ice. It is not necessary to thaw the RT Master Mix (the enzyme is in glycerol). The RT Master Mix should be kept at -20 °C at all times.

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 RNA template) by combining the components of the kit to reach a final volume of 20 µl per reaction (see table 1).

Component	Volume
Taq Master Mix (2x) Blue	10 µl
RT Master Mix (50x) Yellow	0.4 µl
Primers/Probes/IC Mix_PVA/PVM/IC (10x) clear	2 µl
RNA Template / PC / NC	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 RNA template) to each PCR tube or well of an optical-grade PCR plate.

6. Add 2 – 5 µl RNA template to the reaction mix. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the positive control (PC) and the negative control (NC).

7. Seal the tubes or plates, centrifuge briefly to collect components at the bottom of the 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
Reverse Transcription	1	50 oC	20 min
RT inactivation	1	95 oC	5 min
Denaturation Annealing/Extension	40	95 oC 60 oC	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:

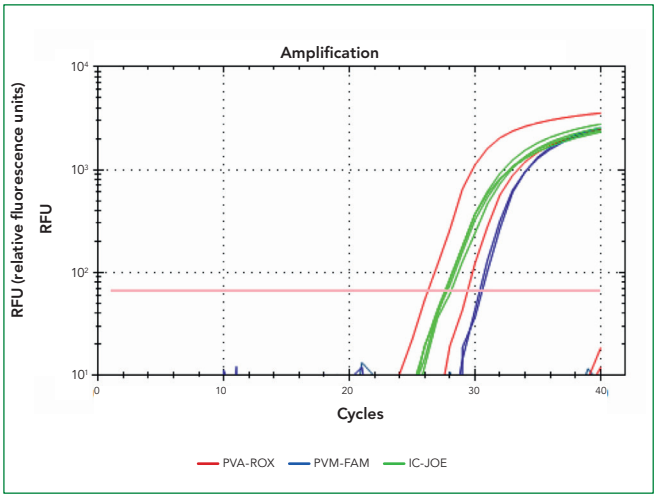
Dye	Virus/Control	Max. EX (nm)	Max. EX (nm)
ROX	PVA	575	602
FAM	PVM	494	515
JOE (HEX Channel)	Internal control (IC)	520	548

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

The graph shows the amplification curves of different PVA- or PVM-infected samples (leaf samples; PVA-ROX in red; PVM-FAM in blue; IC-JOE in green). Healthy control samples and "no template controls" (NTC) show amplification of IC only (green curves).



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.

BIOREBA

Version: 3 – 23.12.2021: Positive control and negative control added as components of the set/kit.

www.bioreba.com

BIOREBA AG
Christoph Merian-Ring 7
CH-4153 Reinach BL1
Switzerland

Your Partner in Agro-Diagnostics

Phone +41 61 712 11 25
Fax +41 61 712 11 17

admin@bioreba.ch
www.bioreba.com