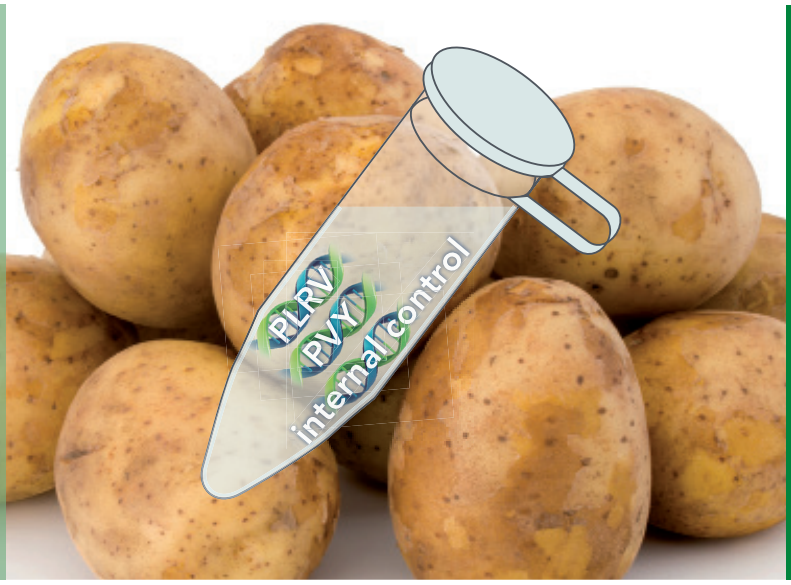
BIOREBA

A CALIBRE SCIENTIFIC COMPANY

qPCR potato leafroll virus (PLRV) / potato virus Y (PVY) set/kit

User Guide



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1. Introduction

1.1 General

The qPCR set enables the simultaneous detection (multiplex) of potato virus Y (PVY; all strains) and potato leafroll virus (PLRV) 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 (PVY: FAM and PLRV: Cy5). 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.
The qPCR set includes a lyophilized PLRV/PVM Lyo RT-qPCR Master Mix 2x which comprises the reverse transriptase (RT), the DNA polymerase as well as the primer and probes.

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. The qPCR for the detection of PLRV, PVY and IC was developed and validated in two certification seasons (2014/15) in collaboration with Agroscope, the Swiss centre of excellence for research in the agriculture and food sectors.

A set and two kits are available for 96 reactions and different pool sizes (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 Y (PVY), potato leaf roll virus (PLRV) 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 condition and quality

3.1 Set format and contents

		Components		
Sales Part No.	**Product name**	**Colour of screw cap**	**Name**	**Volume**
839601	qPCR PLRV/PVY set 96	Blue	PLRV/PVY Lyo RT-qPCR Master Mix (2x) Art. No. 830101	2x 525 µl
		Black	Reconstitution Buffer for Lyo RT-qPCR Art. No. 831414	1.05 ml
		Red	PLRV/PVY qPCR positive control (PC) Art. No. 830054	30 µl
		White	qPCR negative control (NC) Art. No. 830044	30 µl
			Nuclease-free water Art. No. T143.4	1 ml

3.2 Kit format and contents

Part No.	Descripton	No of reactions	Max pool size (potatoes)
839611	**qPCR PLRV/PVY kit 96/10** Includes: • qPCR PLRV/PVY set 96 • Potato DNA/RNA rapid extraction set 96/10x for 96 extractions (Pool size: up to 10 tuber samples)	96	10
839621	**qPCR PLRV/PVY kit 96/25** Includes: • qPCR PLRV/PVY set 96 • Potato DNA/RNA rapid extraction set 96/25x for 96 extractions (Pool size: up to 25 tuber samples)	96	25

3.3 Storage conditions

Store all qPCR components (Lyo RT-qPCR Master Mix, positive control, negative control, Nuclease free Water) at -20 °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 an other 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 on ice or at 4 °C. Thereafter the components should always be kept on ice.

2. 2. Add 525 µl Reconstitution Buffer for Lyo RT-qPCR to the tube with the PLRV/PVY Lyo RT-qPCR Master Mix 2x. Resuspend the lyophilized Master Mix by pipetting up and down until the solution is clear.
Only resuspend as many tubes lyophilized Master Mix as required at the time, since the the Master Mix is most stable in its lyophilized form*.

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

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

5. 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
PLRV/PVY Lyo RT-qPCR Master Mix 2x	10 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

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

7. 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).

8. 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.

*Avoid repeated freeze-thaw cycle of the resuspended Lyo RT-qPCR Master Mix. Unused portion can be divided into aliquots and frozen at -20° C for later use.

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 °C	20 min
RT inactivation	1	95 °C	5 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:

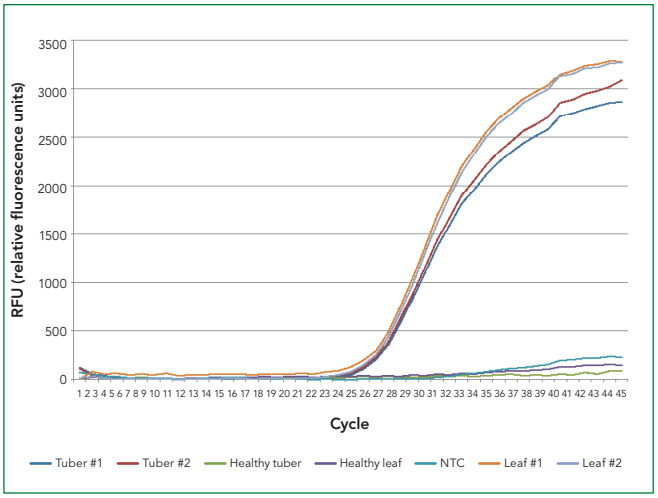
Dye	Virus/Control	Max. Ex (nm)	Max. Em (nm)
Fam	PVY	494	515
Cyanine 5	PLRV	651	674
JOE (HEX Channel)	Internal control (IC)	520	548

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 PVY-infected samples (two leaf samples, two dormant tuber samples). Healthy control samples and "no template controls" (NTC) show no amplification.



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.

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Version: 4 - 27.10.2025 - Changes due to incoorperation of lyophillized enzymes.

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