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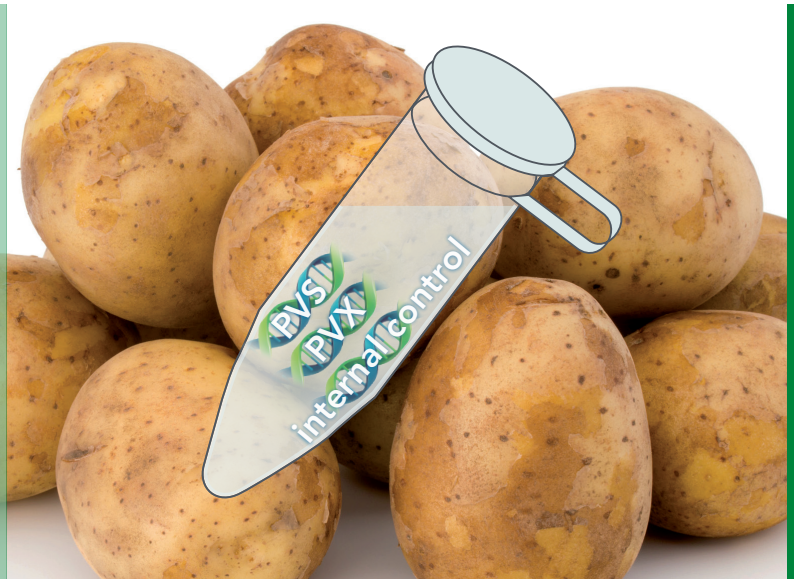
BIOREBA

qPCR potato virus S (PVS)/ potato virus X (PVX) set/kit

User Guide



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1. Introduction

1.1 General

This qPCR set enables the simultaneous detection (multiplex) of potato virus S (PVS) and potato virus X (PVX) 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 (PVS: Cy5 and PVX: 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.
The qPCR PVS/PVX set was developed and validated by BIOREBA. The method is based on the primers published by Schumpp et al., 2021 (1) for the detection of PVX and on primers developed by BIOREBA (M. Kaiser, unpublished) for the detection of PVS. All isolates of PVS tested so far can be detected, including isolates of the Andean strain (PVS^{A}) and isolates of the ordinary strain (PVS^{O}). A full validation report is available on www.bioreba.ch.
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 set" (included in the qPCR kits) for the extraction of RNA. The extraction method with "Potato DNA/RNA rapid extraction set" 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 BIOpick (Art. No. BPS01) from BIOREBA can be used for sampling from potato tubers.
Two qPCR PVS/PVX sets and four kits are available for 96 or 192 extractions and reactions (see page 4 for details).

1.2 Information: PVS and PVX

Potato virus S (PVS), is a single-stranded, positive-sensed RNA virus that is a member of the genus Carlavirus in the family of the *Betaflexiviridae*. PVS has a narrow host range, and susceptible species mainly belong to the *Solanaceae* and *Chenopodiaceae* (e.g. quinoa). PVS can be transmitted by aphids, mechanically, and by vegetative propagation of tubers. PVS is very common in potato fields and has a worldwide distribution. The symptoms caused by PVS are mild and results in minor yield losses in most cases. However, in mixed infection, with PVX, for instance, more severe symptoms may occur (2,3).
Potato Virus X (PVX), is an RNA-virus with a single-stranded, positive-sensed genome, belonging to the Potexviruses in the family of the *Alphaflexiviridae*. PVX is one of the oldest known-potato viruses and is typically transmitted by contaminated farming equipment, or from plant to plant by contact (4). The primary host of PVX is potato, but the virus infects a wide range of dicots. PVX commonly occurs in potato worldwide. It is known to confer severe disease when it occurs in mixed infections with other viruses, especially poty-viruses such as potato virus Y or potato virus A, causing tuber yield losses of up to 80% (3,4).
For testing mixed infections of potato viruses, the same RNA extracts can be used in parallel for testing with BIOREBA qPCR PLRV/PVY set (Art. No. 839610) and BIOREBA qPCR PVA/PVM set (Art. No. 849610).

1.3 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.

1.4 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 S (PVS), potato virus X (PVX) 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**
859600	qPCR PVS/PVX 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_PVS/PVX/IC (10x) Art. No. 850100	0.2 ml
		–	Nuclease-free water Art. No. T143.4	1 ml
		–	PVS and PVX RNA positive control (PC) Art. No. 850053	30 µl
		–	Plant RNA negative control (NC) Art. No. 830043	30 µl
859200	qPCR PVS/PVX 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_PVS/PVX/IC (10x) Art. No. 850100	2 x 0.2 ml
		–	Nuclease-free water Art. No. T143.4	2 x 1 ml
		–	PVS and PVX RNA positive control (PC) Art. No. 850053	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)**
859610	**qPCR PVS/PVX kit 96/10** Includes: • qPCR PVS/PVX set 96 • Potato DNA/RNA rapid extraction set 96/10x for 96 extractions (Pool size: up to 10 tuber samples)	96	10
859625	**qPCR PVS/PVX kit 96/25** Includes: • qPCR PVS/PVX set 96 • Potato DNA/RNA rapid extraction set 96/25x for 96 extractions (Pool size: up to 25 tuber samples)	96	25
859210	**qPCR PVS/PVX kit 192/10** Includes: • qPCR PVS/PVX set 192 • Potato DNA/RNA rapid extraction set 192/10x for 192 extractions (Pool size: up to 10 tuber samples)	192	10
859225	**qPCR PVS/PVX kit 192/25** Includes: • qPCR PVS/PVX 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_PVS/PVX/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 an other DNA/RNA extraction method than the Potato DNA/RNA rapid extraction set 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_PVS/PVX/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 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
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:

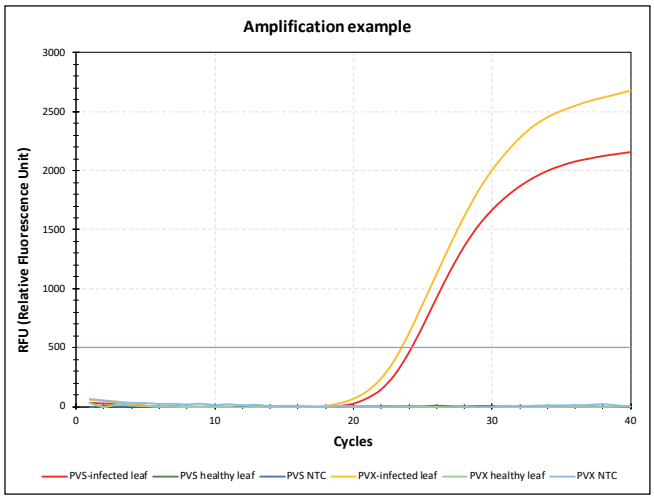
Dye	Virus/Control	Max. EX (nm)	Max. EX (nm)
FAM	PVX	494	515
Cyanine 5	PVS	651	674
JOE (HEX Channel)	Internal control (IC)	520	548

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 PVS and PVX-infected leaf 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.

6. References

(1) Schumpp, O., Brechon, A., Brodard, J. et al. (2021) Large-Scale RT-qPCR Diagnostics for Seed Potato Certification. Potato Research, 64, 553-569.

(2) Chikh-Ali M, Karasev A V. (2023) Virus diseases of potato and their control. Chapter 11 - Potato Production Worldwide, 199-212.

(3) Campos H, Ortiz O. (2019) The potato crop: Its agricultural, nutritional and social contribution to humankind. Springer International Publishing, 1-518.

(4) Verchot J. (2022) Potato virus X: A global potato-infecting virus and type member of the Potexvirus genus. Molecular Plant Pathology, 1;23(3), 315-320.

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