

Validation report

qPCR PVA/PVM set/kits

Article No.: 849600 (qPCR PVA/PVM set 96) / 849200 (qPCR PVA/PVM set 192) /
849610 (qPCR PVA/PVM kit 96/10) / 849625 (qPCR PVA/PVM kit 96/25) /
849210 (qPCR PVA/PVM kit 192/10) / 849225 (qPCR PVA/PVM kit 192/25)

General information:

Target Pathogen	PVA (Potato virus A) / PVM (Potato virus M)
Genus	<i>Potyvirus</i> (PVA) / <i>Carlavirus</i> (PVM)
Method	Real-Time RT-qPCR, TaqMan (Multiplex)

Technical information:

Fluorophores	FAM: PVM ROX: PVA JOE(HEX): IC
Cycling program	➤ 50°C for 20 min (Reverse Transcription) ➤ 95°C for 5 min (RT inactivation) ➤ 40 Cycles: ○ 95°C for 15 sec (Denaturation) ○ 60°C for 30 sec (Annealing / Extension)
Controls	Internal control (IC): artificial RNA included in Primers/Probes/IC Mix Negative control (NC): plant RNA Positive control (PC): RNA from PVA/PVM-infected potatoes
Extraction	The qPCR PVA/PVM kits include the potato DNA/RNA rapid extraction set, which is the validated extraction method for the qPCR PVA/PVM set/kits.

Host matrix:

Tested plant material	Leaf, tuber (dormant), sprouts
Tested species infected	<i>Solanum tuberosum</i> (Potato) <i>Nicotiana tabacum</i> (Tobacco) <i>Physalis peruviana</i> (Cape gooseberry)

Specificity:

Analytical Specificity	100%
Number of tested samples from target organisms (True Positives)	23
Diagnostic Specificity	100%
Number of tested samples non-target organism (True Negatives)	>100
Detected isolates / geographic regions (Inclusivity)	<u>PVA:</u> PVA PV-1266 (Germany, Potato) PVA PV19-159 (Germany, Potato) PVA PV-0535 (Germany, Potato) PVA 775 Charlotte (Switzerland, Potato) PVA 1307 Claustar No. 6 (Switzerland, Potato) PVA Asterix 7778 (UK, Potato) PVA W22-069 (Germany, Potato) <u>PVM:</u> PVM PV-0273 (Germany, Tobacco)

	PVM W19-091 (Germany, Potato) PVM W19-160 (Germany, Potato) PVM BNA Mt. Favet+Mirza (Germany, Potato) PVM Agria (Switzerland, Potato) PVM W22-063 (Germany, Potato)
Cross reaction with (Exclusivity)	None known
No cross reaction tested with (Exclusivity)	PLRV (Potato leafroll virus) PVS (Potato virus S) PVX (Potato virus X) PVY (Potato virus Y)
No matrix effect observed with (Selectivity)	<i>Solanum tuberosum</i> (Potato) – Tuber and Leaf <i>Nicotiana tabacum</i> (Tobacco) <i>Physalis peruviana</i> (Cape gooseberry)

Sensitivity:

Diagnostic Sensitivity	100%
Analytical Sensitivity / LoD	10 ⁻⁵
Sensitivity on host matrix	Potato leaves: 10 ⁻⁵ Potato tubers: 10 ⁻⁵
Other sensitivity characteristics	-

Validation:

Internal validation	2017/2018
External validation	2018 (Switzerland) + proficiency test (PT) every year (Germany) since 2017.
Reproducibility	100%
Repeatability	100%
Validation information	Every year BIOREBA participates with PVA/PVM qPCR kit on an external proficiency test (PT). The kit was in 2018 validated for the testing in seed potato certification (comparison with DAS-ELISA, pools of 10 and 25 tubers are validated).

Validation release Date:
November, 7th, 2022

QC manager:



Version: 2 – 16.05.2024