OREBA

# Validation report qPCR PLRV/PVY set/kits

Article No.:

839600 (qPCR PLRV/PVY set 96) / 839200 (qPCR PLRV/PVY set 192) / 839610 (qPCR PLRV/PVY kit 96/10) / 839625 (qPCR PLRV/PVY kit 96/25) / 839210 (qPCR PLRV/PVY kit 192/10) / 839225 (qPCR PLRV/PVY kit 192/25)

# **General information:**

Target Pathogen	PLRV (Potato leafroll virus) / PVY (Potato virus Y)	
Genus	Polerovirus (PLRV) / Potyvirus (PVY)	
Method	Real-Time RT-qPCR, TaqMan (Multiplex)	

## Technical information:

Fluorophores	FAM: PVY   Cy5: PLRV   JOE(HEX): IC	
Cycling program	50°C for 20 min (Reverse Transcription)	
	95°C for 5 min (RT inactivation)	
	➤ 40 Cycles:	
	<ul> <li>95°C for 15 sec (Denaturation)</li> </ul>	
	<ul> <li>60°C for 30 sec (Annealing / Extension)</li> </ul>	
Controls	Internal control (IC): artificial RNA included in	
	Primers/Probes/IC Mix	
	Negative control (NC): plant RNA	
	Positive control (PC): RNA from PVY/PLRV-infected potatoes	
Extraction	The qPCR PLRV/PVY kits include the potato DNA/RNA rapid	
	extraction set, which is the validated extraction method for	
	the qPCR PLRV/PVY set/kits.	

### Host matrix:

Tested plant material	Leaf, tuber (dormant), sprouts
Tested species infected	Solanum tuberosum (Potato) Nicotiana tabacum "Xanthi" (Tobacco) Physalis floridana (Bladder cherries)

# Specificity:

Analytical Specificity	100%
Number of tested samples from target organisms (True Positives)	>100
Diagnostic Specificity	100%
Number of tested samples non- target organism (True Negatives)	>100
Detected isolates / geographic regions (Inclusivity)	PLRV:PV-0842 (Germany, Bladder cherries)W19-160 (Germany, Potato)1 (Switzerland, Bladder cherries)Agata (Switzerland Potato)Valor 4 (Australia, Potato)PVY:W19-094 (Germany, Potato)W19-095 (Germany, Potato)PV-0343 (Germany, Potato)

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	N-605 (Switzerland, Tobacco) N-605 Victoria (Switzerland, Potato) O-803 (Switzerland, Tobacco) O-768 1118 Victoria (Switzerland, Potato) NTN 1317 Charlotte (Switzerland, Potato) NTN 1214 Victoria (Switzerland, Potato)	
Cross reaction with (Exclusivity)	None known	
No cross reaction tested with (Exclusivity)	PVA (Potato virus A) PVM (Potato virus M) PVS (Potato virus S) PVX (Potato virus X)	
No matrix effect observed with (Selectivity)	Solanum tuberosum (Potato) – Tuber and Leaf Nicotiana tabacum "Xanthi" (Tobacco) Physalis floridana (Bladder cherries)	

#### Sensitivity:

Analytical Sensitivity / LoD	10 <sup>-5</sup> to 10 <sup>-6</sup>
Sensitivity on host matrix	Potato leaves: 10 <sup>-5</sup> to 10 <sup>-6</sup> Potato tubers: 10 <sup>-5</sup> to 10 <sup>-6</sup>
Other sensitivity characteristics	-

#### Validation:

Internal validation	2014/2015 + 2016/2017
External validation	2015 (Switzerland), 2017 (Switzerland) and proficiency test (PT) every year (Germany) since 2017.
Reproducibility	100%
Repeatability	100%
	Every year BIOREBA participates with PLRV/PVY qPCR kit on an external proficiency test (PT).
Validation information	The kit was in 2016/2017 validated (using 94 potato lots of 200 tubers each) for the testing in seed potato certification (comparison with DAS-ELISA, pools of 10 and 25 tubers are validated).

Validation release Date: November, 7<sup>th</sup>, 2022

QC manager:

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