



AmplifyRP® XRT for PMTV
Validation Report
Potato mop-top virus
Product No. XCS 12501



Test Characteristics

Test Name	Potato mop-top virus	Test Label	FAM-labeled target probe
Catalog Number	12501	Internal Control	Endogenous
Acronym	PMTV	Format	XRT
Genus	Pomovirus	Diluents	GEB/PD1
Binomial Name	Pomovirus solani	Sample Dilution	1:10

Summary

AmplifyRP XRT for PMTV is a rapid RNA amplification and detection platform designed for testing potato leaves, stems, tubers, or tissue culture plantlets for Potato mop-top virus. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify PMTV RNA and an endogenous RNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	30
Correct Diagnoses	30
Percent	100%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 87.5% sensitive between 1 fg/μL and 10 fg/μL. (n=16)
Limit of Detection:	The assay has a 100% detection rate at 10 fg/μL with RNA transcripts. (n=8)
	The assay has a 75% detection rate at 1 fg/μL with RNA transcripts. (n=8)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

PMTV-23T (Colombia) ¹	PMTV-C52_P11 (Peru) ¹
PMTV-Guangdong (China) ¹	PMTV-ID-Idaho_1 (USA) ¹
PMTV-J20_P157 (Peru) ¹	PMTV-J21_P117 (Peru) ¹
PMTV-Maryland (USA) ¹	PMTV-Nd4 (Canada) ¹
PMTV-physalis_M7 (Colombia) ¹	PMTV-PV-0582 (UK)
PMTV-quitoense_M6 (Colombia) ¹	PMTV-Swedish, (Sw) (Sweden) ¹
PMTV-Todd ¹	PMTV-Yunnan (China) ¹
¹ Predicted detection by in silico analysis only	

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
Alfalfa mosaic virus (AMV)	Alfavirus AMV
Andean potato latent virus (APLV) ¹	Tymovirus latandigenum
Andean potato mottle virus (APMoV)	Comovirus andesense

Does Not Cross-react With:

Virus Name	Species Name
Barley stripe mosaic virus (BSMV) ¹	Hordeivirus hordei
Beet soil-borne virus (BSBV)	Pomovirus solibetae
Beet virus Q (BVQ) ¹	Pomovirus betae
Broad bean necrosis virus (BBNV) ¹	Pomovirus viciae
Colombian potato soil-borne virus (CPSbV) ¹	Pomovirus colombiense
Cucumber green mottle mosaic virus (CGMMV) ¹	Tobamovirus viridimaculae
Indian peanut clump virus (IPCV) ¹	Pecluvirus indicum
Japanese soil-borne wheat mosaic virus (JSBWMV) ¹	Furovirus japonicum
Pea early-browning virus (PEBV) ¹	Tobravirus pisi
Peanut clump virus (PCV) ¹	Pecluvirus arachidis
Pepper ringspot virus (PepRSV) ¹	Tobravirus capsici
Potato aucuba mosaic virus (PAMV)	Potexvirus marmoraucuba
Potato latent virus (PotLV)	Carlavirus latensolani
Potato leafroll virus (PLRV)	Polerovirus PLRV
Potato virus A (PVA)	Potyvirus atuberosi
Potato virus M (PVM)	Carlavirus misolani
Potato virus S (PVS)	Carlavirus sigmasolani
Potato virus T (PVT)	Tepovirus tafsolani
Potato virus V (PVV)	Potyvirus vetuberosi
Potato virus X (PVX)	Potexvirus ecspotati
Potato virus Y (PVY)	Potyvirus yituberosi
Rehmannia mosaic virus (ReMV) ¹	Tobamovirus rehmanniae
Ribgrass mosaic virus (RMV) ¹	Tobamovirus plantagonis
Soil-borne wheat mosaic virus (SBWMV) ¹	Furovirus tritici
Tobacco etch virus (TEV)	Potyvirus nicotianainsculptentis
Tobacco mild green mosaic virus (TMGMV) ¹	Tobamovirus mititessellati
Tobacco mosaic virus (TMV) ¹	Tobamovirus tabaci
Tobacco rattle virus (TRV)	Tobravirus tabaci
Tomato brown rugose fruit virus (ToBRFV) ¹	Tobamovirus fructirugosum
Tomato mosaic virus (ToMV) ¹	Tobamovirus tomatotessellati
Tomato spotted wilt virus (TSWV)	Orthotospovirus tomatomaculae

¹Predicted non-detection by in silico analysis only

Diagnostic Specificity

True Negatives 66
Correct Diagnoses 66
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Potato leaves	Potato sprouts	Potato tubers	
The hosts on the above list have been chosen to represent those which historically cause a range of matrix effects, in addition to those expected to be screened for this pathogen. Not all plant species susceptible to this pathogen have been screened, but may still be used with this assay unless otherwise noted below. As with all diagnostic tools, Agdia recommends confirming all results with a secondary detection method before making any economic decisions (ex: discarding plants due to positive test results, etc.).			

Matrix Effect Observed With:			
None Known			

Repeatability

Number of Samples 88
Replicates per Sample 2 - 3
Total Replicates 190
Replicates in Agreement 187
Percent Agreement 98.4%

Reproducibility

Number of Samples 33
Replicates per Sample 2 - 3
Number of Operators 3
Total Replicates 198
Replicates in Agreement 182
Percent Agreement 91.9%

Robustness

Planned deviation analysis:

No deviations from the user guide protocol were validated.

Stability:

	1-year stability (accelerated)	Real-time Stability Verification
Positive Sample (High)	Pass	Monitoring
Positive Sample (High)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Negative Sample	Pass	Monitoring
Negative Sample	Pass	Monitoring

Glossary

Diagnostic sensitivity¹:	The percentage of positive samples correctly identified in an experiment with known positive controls.
Diagnostic specificity¹:	The percentage of negative samples correctly identified in an experiment with known negative controls.
Analytical sensitivity³:	The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')
Analytical specificity²:	(comprises inclusivity and exclusivity)
Inclusivity³:	The performance of a test with a range of target isolates covering genetic diversity, different geographical origin and/or hosts associated with the target organism.
Exclusivity³:	The performance of a test with a range of non-targets (e.g. cross-reaction with closely related organisms, contaminants)
Selectivity²:	The level of effect that matrices and relevant plant parts have on the performance of the assay.
Repeatability²:	The agreement between test replicates of the same sample tested by the same operator.
Reproducibility³:	The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, users, equipment, location)
Robustness^{1,3}:	The extent to which varying test conditions (e.g. temperature, volume, change of buffers) affect the established test performance values. May also be referred to as planned deviation analysis.
Stability¹:	The performance of test reagents or controls over time.

References:

¹Groth-Helms, D., Rivera, Y., Martin, F. N., Arif, M., Sharma, P., Castlebury, L. A. (in press). Terminology and Guidelines for Diagnostic Assay Development and Validation: Best Practices for Molecular Tests. *PhytoFrontiers*.

²Eads, A., Groth-Helms, D., Davenport, B., Cha, X., Li, R., Walsh, C., Schuetz, K., (in press). The Commercial Validation of Three Tomato Brown Rugose Fruit Virus Assays. *PhytoFrontiers*.

³EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, EPPO Bulletin 48, 373– 377.

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AmplifyRP Test Kits employ recombinase polymerase amplification (RPA) technology, developed by TwistDx Limited, U.K. Use of the RPA process and probe technologies are protected by US patents 7,270,981 B2, 7,399,590 B2, 7,435,561 B2, 7,485,428 B2 and foreign equivalents in addition to pending patents.

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