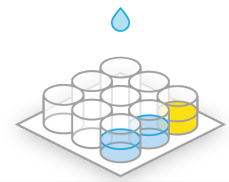


Validation Report: ELISA

PSA/SRA 31505 • Plum pox virus (PPV)



Test Characteristics

Test Name	Plum pox virus	Capture Antibody	Polyclonal (Rabbit)
Catalog Number	31505	Detection Antibody	Polyclonal (Rabbit)
Acronym	PPV	Format	DAS-ELISA
Genus	Potyvirus	Diluents	GEB4/RUB3
Binomial Name	Potyvirus plumpoxi	Sample Dilution	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Plum pox virus (PPV) in stone fruit leaves. PPV is a member of the Potyvirus genus known for their non-enveloped, flexuous, filamentous virus particles.

Diagnostic Sensitivity

True Positives	107
Correct Diagnoses	104
Percent	97.2%

Analytical Sensitivity

Limit of Detection: 1:40,000 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

PPV-An (Marcus ancestor) (Albania)	PPV-C (Cherry) (Moldova)
PPV-CR (Cherry Russia) (Russia)	PPV-D (Dideron)
PPV-EA (El Amar) (Egypt)	PPV-M (Marcus)
PPV-Rec (Recombinant) (Serbia)	PPV-SoC (Sour Cherry) (Moldova)
PPV-T (Turkey)	PPV-W (Winona) (Canada)

Exclusivity:

Cross-reacts With:

Potyvirus alstromeriae (Alstroemeria mosaic virus) (AIMV)	Potyvirus perulycopersici (Peru tomato mosaic virus) (PTV)
Potyvirus vetuberosi (Potato virus V) (PVV)	Potyvirus gebataatae (Sweet potato virus G) (SPVG)

Does Not Cross-react With:

Ampelovirus nanoavii (Little cherry virus 2) (LChV2)	Foveavirus latensarmeniacaе (Apricot latent virus) (ALV)
Illarvirus APLPV (American plum line pattern virus) (APLPV)	Illarvirus ApMV (Apple mosaic virus) (ApMV)
Illarvirus PDV (Prune dwarf virus) (PDV)	Illarvirus PNRSV (Prunus necrotic ringspot virus) (PNRSV)
Nepovirus arabis (Arabis mosaic virus) (ArMV)	Nepovirus avii (Cherry leaf roll virus) (CLRV)



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Does Not Cross-react With:

Nepovirus lycopersici (Tomato ringspot virus) (ToRSV)	Nepovirus nicotianae (Tobacco ringspot virus) (TRSV)
Nepovirus nigranuli (Tomato black ring virus) (TBRV)	Nepovirus persicae (Peach rosette mosaic virus) (PRMV)
Potyvirus algeriaense (Watermelon mosaic virus) (WMV)	Potyvirus ampeloprasi (Leek yellow stripe virus) (LYSV)
Potyvirus arachidis (Peanut mottle virus) (PeMoV)	Potyvirus ascaloniae (Shallot yellow stripe virus) (SYSV)
Potyvirus atuberosi (Potato virus A) (PVA)	Potyvirus betaceum (Beet mosaic virus) (BtMV)
Potyvirus capsimaculae (Pepper mottle virus) (PepMoV)	Potyvirus cepae (Onion yellow dwarf virus) (OYDV)
Potyvirus citrullimoroccense (Moroccan watermelon mosaic virus) (MWMV)	Potyvirus cucurbitaflaviteselati (Zucchini yellow mosaic virus) (ZYMV)
Potyvirus dasheenii (Dasheen mosaic virus) (DsMV)	Potyvirus dianthi (Carnation vein mottle virus) (CarMV)
Potyvirus glycitessellati (Soybean mosaic virus) (SMV)	Potyvirus halapensis (Johnsongrass mosaic virus) (JGMV)
Potyvirus lactucae (Lettuce mosaic virus) (LMV)	Potyvirus musae (Banana bract mosaic virus) (BBrMV)
Potyvirus nicotianainsculpentis (Tobacco etch virus) (TEV)	Potyvirus nicotianavenamaculae (Tobacco vein mottling virus) (TVMV)
Potyvirus papayanuli (Papaya ringspot virus) (PRSV)	Potyvirus phaseoliteum (Bean yellow mosaic virus) (BYMV)
Potyvirus phaseovulgaris (Bean common mosaic virus) (BCMV)	Potyvirus pisumsemenportati (Pea seed-borne mosaic virus) (PSbMV)
Potyvirus rapae (Turnip mosaic virus) (TuMV)	Potyvirus sacchari (Sugarcane mosaic virus) (SCMV)
Potyvirus trifolii (Clover yellow vein virus) (CIYVV)	Potyvirus wisteriae (Wisteria vein mosaic virus) (WVMV)
Potyvirus yituberosi (Potato virus Y) (PVY)	Potyvirus zeananus (Maize dwarf mosaic virus) (MDMV)
Stralirivirus fragariae (Strawberry latent ringspot virus) (SLRSV)	Trichovirus mali (Apple chlorotic leaf spot virus) (ACLSV)

Diagnostic Specificity

True Negatives 208
 Correct Diagnoses 208
 Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Almond leaves	Almond petioles	Apricot fruit	Apricot leaves
Apricot petioles	Apricot skin	Cherry fruit	Cherry leaves
Cherry petioles	Cherry skin	Nectarine fruit	Nectarine leaves
Nectarine petioles	Nectarine skin	Peach buds	Peach fruit
Peach leaves	Peach petioles	Peach skin	Plum buds
Plum fruit	Plum leaves	Plum petioles	Plum skin

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			
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Repeatability

Number of Samples 315
Replicates per Sample 3 - 9
Total Replicates 1941
Replicates in Agreement 1924
Percent Agreement 99.1%

Reproducibility

Number of Samples 32
Replicates per Sample 6
Number of Operators 3
Total Replicates 576
Replicates in Agreement 576
Percent Agreement 100%

Robustness

Planned deviation analysis:

	Average OD Values			
	O.N. coating / 2 hour sample	4 hour coating / 2 hour sample	4 hour coating / O.N. sample	O.N. coating / O.N. sample
Positive Sample #1 (High)	1.257	1.147	1.955	2.082
Positive Sample #1 (Low)	0.304	0.285	0.537	0.589
Positive Sample #2 (High)	1.218	0.905	1.571	2.060
Positive Sample #2 (Low)	0.318	0.267	0.483	0.701
Positive Sample #3 (High)	3.125	2.726	3.562	3.740
Negative Sample #1	0.092	0.091	0.085	0.096
Negative Sample #2	0.094	0.092	0.093	0.097
Negative Sample #3	0.094	0.089	0.090	0.106
Negative Sample #4	0.092	0.090	0.096	0.111
Negative Sample #5	0.095	0.091	0.093	0.101
Buffer	0.091	0.093	0.092	0.088

Stability:

	1-year stability (accelerated)	1-year stability (real time)
Positive Sample (High)	Pass	Monitoring
Positive Sample (Medium)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Negative Sample #1	Pass	Monitoring
Negative Sample #2	Pass	Monitoring
Buffer	Pass	Monitoring
Negative Control	Pass	Monitoring



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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 sensitivity²:	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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