AmplifyRP® XRT for FD
Validation Report
Phytoplasma vitis Flavescence Doree
Product No. XCS 77300

Test Characteristics

Test Name	Phytoplasma vitis Flavescence Doree	**Test Label**	FAM-labeled target probe
Catalog Number	77300	**Internal Control**	Endogenous
Acronym	FD	**Format**	XRT
Genus	Phytoplasma	**Diluents**	GEB/PD1
Binomial Name	Candidatus Phytoplasma vitis	**Sample Dilution**	1:20

Summary

AmplifyRP XRT for FD is a rapid DNA amplification and detection platform designed for testing grapevines for Phytoplasma vitis Flavescence Dorée. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify FD DNA and an endogenous DNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	39
Correct Diagnoses	39
Percent	100%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 80% sensitive between 10 ag/µL and 100 ag/µL. (n=10)
Limit of Detection:	The assay has a 100% detection rate at 100 ag/µL with DNA fragments. (n=5)
	The assay has a 60% detection rate at 10 ag/µL with DNA fragments. (n=5)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

FD-ALY (Italy)	FD-FD-2000 (France)
FD-FD-70 (France)	FD-FD-92
FD-FD-C (Italy)	FD-FD-CCI (Italy)
FD-FD-CS (Serbia)	FD-FD-D (Italy)
FD-FD-PS (France)	

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
Hemp dogbane phytoplasma[1]	Palatinate Grapevine Yellows[1]
Phytoplasma asteris[1]	Phytoplasma australiense[1]
Phytoplasma balanitae[1]	Phytoplasma pruni[1]
Phytoplasma rubi[1]	Phytoplasma solani
Phytoplasma trifolii[1]	Phytoplasma ulmi[1]
Phytoplasma ziziphi[1]	
[1]Predicted non-detection by *in silico* analysis only	

Diagnostic Specificity

True Negatives	29
Correct Diagnoses	29
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Grape canes	Grape leaves	Grape petioles	
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	16
Replicates per Sample	2
Total Replicates	32
Replicates in Agreement	32
Percent Agreement	100%

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 sensitivity[1]:	The percentage of positive samples correctly identified in an experiment with known positive controls.
Diagnostic specificity[1]:	The percentage of negative samples correctly identified in an experiment with known negative controls.
Analytical sensitivity[3]:	The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')
Analytical specificity[3]:	(comprises inclusivity and exclusivity)
Inclusivity[3]:	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.
Exclusivity[3]:	The performance of a test with a range of non-targets (e.g. cross-reaction with closely related organisms, contaminants)
Selectivity[2]:	The level of effect that matrices and relevant plant parts have on the performance of the assay.
Repeatability[2]:	The agreement between test replicates of the same sample tested by the same operator.
Reproducibility[3]:	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.
Stability[1]:	The performance of test reagents or controls over time.

References:

[1]Groth-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.
[2]Eads, 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.
[3]EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, EPPO Bulletin 48, 373– 377.

Questions or Technical Support:

Phone: 800-622-4342 (toll-free) or 574-264-2014
Fax: 574-264-2153
E-mail: info@agdia.com for sales and general product information
techsupport@agdia.com for technical information and troubleshooting

Web: www.agdia.com