AmplifyRP® XRT for GLRaV-3
Validation Report
Grapevine leafroll-associated virus 3
Product No. XCS 29001

Test Characteristics

Test Name	Grapevine leafroll-associated virus 3	**Test Label**	FAM-labeled target probe
Catalog Number	29001	**Internal Control**	Endogenous
Acronym	GLRaV-3	**Format**	XRT
Genus	Ampelovirus	**Diluents**	GEB/PD1
Binomial Name	Ampelovirus trivitis	**Sample Dilution**	1:10

Summary

AmplifyRP XRT for GLRaV-3 is a rapid RNA amplification and detection platform designed for testing grapevines for Grapevine leafroll-associated virus 3. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify GLRaV-3 RNA and an endogenous RNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	74
Correct Diagnoses	73
Percent	98.6%

Analytical Sensitivity

Analytical Sensitivity: The assay is 75% sensitive between 1.25 fg/µL and 2.5 fg/µL. (n=12)

Limit of Detection: The assay has a 100% detection rate at 2.5 fg/µL with RNA transcript. (n=6)

The assay has a 50% detection rate at 1.25 fg/µL with RNA transcript. (n=6)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

GLRaV-3-185 (Group V)[1]	GLRaV-3-8415A (Canada)
GLRaV-3-AU173 (Group I) (Spain)[1]	GLRaV-3-BHA172 (Group I) (Spain)[1]
GLRaV-3-GH11(Group VI) (South Africa)	GLRaV-3-GH24 (Group VII) (South Africa)
GLRaV-3-Gor259 (Group III) (France)[1]	GLRaV-3-Kat255 (Group I) (Canada)[1]
GLRaV-3-Kis252 (Group I) (Hungary)[1]	GLRaV-3-LN3204 (Group I) (USA)
GLRaV-3-LR3f (Group IX) (USA)[1]	GLRaV-3-NdA121 (Group VII) (Italy)
GLRaV-3-NZ2 (Group X) (New Zealand)[1]	GLRaV-3-Pin244b (Group IX) (USA)
GLRaV-3-Pro95 (Group IX) (USA)[1]	GLRaV-3-TC-BR (Group II) (Brazil)[1]
GLRaV-3-Tou260 (Group I) (Portugal)[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
Arabis mosaic virus (ArMV)	Nepovirus arabis
Grapevine fanleaf virus (GFLV)	Nepovirus foliumflabelli
Grapevine fleck virus (GFkV)	Maculavirus vitis
Grapevine leafroll-associated virus 1 (GLRaV-1)	Ampelovirus univitis
Grapevine leafroll-associated virus 2 (GLRaV-2)	Closterovirus vitis
Grapevine leafroll-associated virus 4 (GLRaV-4)	Ampelovirus tetravitis
Grapevine leafroll-associated virus 7 (GLRaV-7)	Velarivirus septemvitis
Grapevine pinot gris virus (GPGV)	Trichovirus pinovitis
Grapevine red blotch virus (GRBV)	Grablovirus vitis
Little cherry virus 2 (LChV2)	Ampelovirus nanoavii
N/A	Botrytis cinerea (Bcin)
N/A	Phytoplasma solani BN (Bois Noir)
N/A	Phytoplasma vitis FD (Flavescence Dorée)
N/A	Xylella fastidiosa (Xf)
Peach rosette mosaic virus (PRMV)	Nepovirus persicae
Pineapple mealybug wilt associated virus-2 (PMWaV-2)[1]	Ampelovirus duananas
Pineapple mealybug wilt associated virus-3 (PMWaV-3)[1]	Ampelovirus triananas
Pineapple mealybug wilt-associated virus 1 (PMWaV-1)[1]	Ampelovirus unananas
Plum bark necrosis stem pitting-associated virus (PBNSPaV)[1]	Ampelovirus pruni
Strawberry latent ringspot virus (SLRSV)[1]	Stralarivirus fragariae
Tobacco ringspot virus (TRSV)	Nepovirus nicotianae
Tomato ringspot virus (ToRSV)	Nepovirus lycopersici
[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 leaves	Grape petioles	Grape stems	
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	67
Replicates per Sample	2 - 3
Total Replicates	136
Replicates in Agreement	135
Percent Agreement	99.3%

Reproducibility

Number of Samples	23
Replicates per Sample	1
Number of Operators	3
Total Replicates	69
Replicates in Agreement	68
Percent Agreement	98.6%

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