



AmplifyRP® XRT for WCLaV-1,2
Validation Report
Watermelon crinkle leaf-associated virus 1 & 2
Product No. XCS 39800



Test Characteristics

Test Name	Watermelon crinkle leaf-associated virus 1 & 2	Test Label	FAM-labeled target probe
Catalog Number	39800	Internal Control	N/A
Acronym	WCLaV-1,2	Format	XRT
Genus	Coguvirus	Diluents	GEB2/PD1
Binomial Name	Coguvirus citrulli (WCLaV-1) Coguvirus henanense (WCLaV-2)	Sample Dilution	1:20

Summary

AmplifyRP® XRT for WCLaV-1,2 is a rapid RNA amplification and detection platform designed for testing cucurbits for Watermelon crinkle leaf-associated virus 1 & 2. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify WCLaV-1,2 RNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	186
Correct Diagnoses	182
Percent	97.8%

Analytical Sensitivity

WCLaV-1	Analytical Sensitivity:	The assay is 81.3% sensitive between 500 ag/μL and 1 fg/μL. (n=16)
	Limit of Detection:	The assay has a 100% detection rate at 1 fg/μL with RNA transcripts. (n=8) The assay has a 62.5% detection rate at 500 ag/μL with RNA transcripts. (n=8)
WCLaV-2	Analytical Sensitivity:	The assay is 75.0% sensitive between 10 fg/μL and 100 fg/μL. (n=32)
	Limit of Detection:	The assay has a 100% detection rate at 100 fg/μL with RNA transcripts. (n=8) The assay has a 50.0% detection rate at 10 fg/μL with RNA transcripts. (n=24)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

WCLaV-1 USA isolate	WCLaV-1-Ju-01 (Brazil) ¹
WCLaV-1-KF-1 (China) ¹	WCLaV-1-S6 (Australia) ¹
WCLaV-2 USA isolate	WCLaV-2-Ju-01 (Brazil) ¹
WCLaV-2-KF-15 (China) ¹	
¹ Predicted detection by <i>in silico</i> analysis only	

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
Blackberry line pattern virus (BlaLPV) ¹	Coguvirus rubi
Brassica campestris chinensis coguvirus 1 (BCCoV1) ¹	Coguvirus chinense
Citrus concave gum-associated virus (CCGaV) ¹	Coguvirus citri
Citrus virus A (CiVA) ¹	Coguvirus eburni
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucurbit chlorotic yellows virus (CCYV) ²	N/A
Cucurbit leaf crumple virus (CuLCrV) ¹	Begomovirus cucurbitae
Cucurbit yellow stunting disorder virus (CYSDV)	Crinivirus cucurbitae
Edgeworthia chrysantha mosaic-associated virus (ECMaV) ¹	Coguvirus chrysanthae
Melon necrotic spot virus (MNSV)	Gammacarmovirus melonis
Papaya ringspot virus (PRSV)	Potyvirus papayanuli
Squash mosaic virus (SqMV)	Comovirus cucurbitae
Squash vein yellowing virus (SqVYV)	Ipomovirus cucurbitavenaflavi
Watermelon mosaic virus (WMV)	Potyvirus citrulli
Yúnnán Paris negative-stranded virus (YPNSV) ¹	Coguvirus yunnanense
Zucchini yellow mosaic virus (ZYMV)	Potyvirus cucurbitaflaviteselati
¹ Predicted non-detection by <i>in silico</i> analysis only	
² Described by ICTV as a related, unclassified Crinivirus	

Diagnostic Specificity

True Negatives 69
 Correct Diagnoses 69
 Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Bitter gourd leaves	Bitter gourd petioles	Bitter gourd stems	Bottle gourd leaves
Bottle gourd petioles	Bottle gourd stems	Cucumber leaves	Cucumber petioles
Cucumber seeds	Cucumber stems	Hemp leaves	Hemp petioles
Hemp stems	Melon leaves	Melon petioles	Melon stems
Pumpkin leaves	Pumpkin petioles	Pumpkin seeds	Pumpkin stems
Squash leaves	Squash petioles	Squash stems	Watermelon leaves
Watermelon rinds	Watermelon seeds	Zucchini leaves	Zucchini petioles
Zucchini 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	255
Replicates per Sample	2 - 8
Total Replicates	546
Replicates in Agreement	532
Percent Agreement	97.4%

Reproducibility

Number of Samples	24
Replicates per Sample	3
Number of Operators	4
Total Replicates	288
Replicates in Agreement	288
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 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.

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

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.

AmplifyRP® is a registered trademark of Agdia, Inc.