



AmplifyRP® XRT for CYSDV
Validation Report
Cucurbit yellow stunting disorder virus
Product No. XCS 72699



Test Characteristics

Test Name	Cucurbit yellow stunting disorder virus	Test Label	FAM-labeled target probe
Catalog Number	72699	Internal Control	N/A
Acronym	CYSDV	Format	XRT
Genus	Crinivirus	Diluents	GEB2/PD1
Binomial Name	Crinivirus cucurbitae	Sample Dilution	1:20

Summary

AmplifyRP XRT for CYSDV is a rapid RNA amplification and detection platform designed for testing cucurbits for Cucurbit yellow stunting disorder virus. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify CYSDV RNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	147
Correct Diagnoses	143
Percent	97.3%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 58.4% sensitive between 5 fg/μL and 10 fg/μL. (n=13)
Limit of Detection:	The assay has a 100% detection rate at 10 fg/μL with RNA transcripts. (n=7)
	The assay has a 16.7% detection rate at 5 fg/μL with purified virus. (n=6)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CYSDV California, USA isolate	CYSDV Lebanon isolate
CYSDV-14C (GA, USA) ¹	CYSDV-AILM (Spain) ¹
CYSDV-Arizona-1 (USA) ¹	CYSDV-CaF2A2019 (GA, USA) ¹
CYSDV-CF2A-524_1 (GA, USA) ¹	CYSDV-Iraq-1 (Iraq) ¹
CYSDV-KB (Thailand) ¹	CYSDV-PV-0590 (Morocco)
CYSDV-PV-0591 (Spain)	CYSDV-PV-1144 (Greece)
CYSDV-Saladin (Iraq) ¹	CYSDV-Spanish (Spain) ¹

¹Predicted 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
Abutilon yellows virus (AbYV) ¹	Crinivirus abutilonis
Alfalfa mosaic virus (AMV)	Alfavirus AMV
Bean yellow disorder virus (BnYDV)	Crinivirus flavibetae
Beet pseudoyellows virus (BPYV)	Crinivirus pseudobetae
Blackberry yellow vein-associated virus (BYVaV) ¹	Crinivirus rubi
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucurbit chlorotic yellows virus (CCYV) ²	N/A
Diodia vein chlorosis virus (DVCV)	Crinivirus diodiae
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri
Lettuce chlorosis virus (LCV)	Crinivirus lactucachlorosi
Lettuce infectious yellows virus (LIYV)	Crinivirus lactucaflavi
Melon necrotic spot virus (MNSV)	Gammacarmovirus melonis
Papaya ringspot virus (PRSV)	Potyvirus papayanuli
Potato yellow vein virus (PYVV)	Crinivirus flavisolani
Squash mosaic virus (SqMV)	Comovirus cucurbitae
Strawberry pallidosis-associated virus (SPaV)	Crinivirus palidofragariae
Sweet potato chlorotic stunt virus (SPCSV)	Crinivirus ipomeae
Tetterwort vein chlorosis virus (TwVVCV)	Crinivirus chelidonii
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato chlorosis virus (ToCV)	Crinivirus tomatichlorosis
Tomato infectious chlorosis virus (TICV)	Crinivirus contagichlorosis
Tomato leaf curl New Delhi virus (ToLCNDV)	Begomovirus solanumdelhiense
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatusellati
Watermelon mosaic virus (WMV)	Potyvirus citrulli
Zucchini green mottle mosaic virus (ZGMMV)	Tobamovirus cucurbitae
Zucchini yellow mosaic virus (ZYMV)	Potyvirus cucurbitaflavitesellati
¹ Predicted non-detection by <i>in silico</i> analysis only	
² Described by ICTV as a related, unclassified Crinivirus	

Diagnostic Specificity

True Negatives 108
 Correct Diagnoses 108
 Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Bean leaves	Bean petioles	Bean stems	Bitter gourd leaves
Bitter gourd petioles	Bitter gourd stems	Bottle gourd leaves	Bottle gourd petioles
Bottle gourd stems	Cucumber leaves	Cucumber petioles	Cucumber stems
Ground cherry leaves ¹	Ground cherry petioles ¹	Ground cherry stems	Hemp leaves ¹
Hemp petioles ¹	Hemp stems	Lettuce leaves	Melon leaves
Melon petioles	Melon stems	Pumpkin leaves	Pumpkin petioles

No Matrix Effect Observed With:			
Pumpkin stems	Squash leaves	Squash petioles	Squash stems
Tomato leaves	Tomato petioles	Tomato stems	Watermelon leaves
Watermelon petioles	Watermelon stems		
¹ A 10-fold reduction in sensitivity has been observed due to inhibition.			
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:			
Ground cherry leaves ¹	Ground cherry petioles ¹	Hemp leaves ¹	Hemp petioles ¹
¹ A 10-fold reduction in sensitivity has been observed due to inhibition.			

Repeatability

Number of Samples	254
Replicates per Sample	2 - 7
Total Replicates	650
Replicates in Agreement	632
Percent Agreement	97.2%

Reproducibility

Number of Samples	24
Replicates per Sample	3
Number of Operators	4
Total Replicates	288
Replicates in Agreement	286
Percent Agreement	99.3%

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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