AmplifyRP® XRT for CCYV
Validation Report
Cucurbit chlorotic yellows virus
Product No. XCS 41400

Test Characteristics

Test Name	Cucurbit chlorotic yellows virus	**Test Label**	FAM-labeled target probe
Catalog Number	41400	**Internal Control**	N/A
Acronym	CCYV	**Format**	XRT
Genus	Crinivirus	**Diluents**	GEB2/PD1
Binomial Name	Unclassified Crinivirus	**Sample Dilution**	1:20

Summary

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

Diagnostic Sensitivity

True Positives	79
Correct Diagnoses	79
Percent	100%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 75.0% sensitive between 100 ag/µL and 1 fg/µL. (n=20)
Limit of Detection:	The assay has a 100% detection rate at 1 fg/µL with RNA transcripts. (n=10)
	The assay has a 50.0% detection rate at 100 ag/µL with RNA transcripts. (n=10)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CCYV Lebanon Isolate	CCYV-AB523788 (Japan)[1]
CCYV-Beijing (China)[1]	CCYV-CaF1-523_1 (GA, USA)[1]
CCYV-cannabis1 (Israel)[1]	CCYV-CC-XH (China)[1]
CCYV-CF1-525_1 (GA, USA)[1]	CCYV-C-JJ2 (South Korea)[1]
CCYV-C-NS6 (South Korea)[1]	CCYV-Cume-533-21 (Italy)[1]
CCYV-DSMZ PV-1020 (Sudan)[1]	CCYV-ES-1 (South Korea)[1]
CCYV-ES-2 (South Korea)[1]	CCYV-ES-3 (South Korea)[1]
CCYV-ES-4 (South Korea)[1]	CCYV-GDLJ (China)[1]
CCYV-GDNS (China)[1]	CCYV-GDXW (China)[1]
CCYV-Georgia (USA)[1]	CCYV-HeNan (China)[1]
CCYV-IL (Israel)[1]	CCYV-Imperial Valley (CA, USA)
CCYV-KY618798 (Taiwan)[1]	CCYV-M-JJU1 (South Korea)[1]
CCYV-M-UR27 (South Korea)[1]	CCYV-pCCYCRNA13 (China)[1]
CCYV-P-GY2 (South Korea)[1]	CCYV-Rg (China)[1]
CCYV-SD (China)[1]	CCYV-ShangHai (China)[1]
CCYV-Sqworth (GA, USA)[1]	CCYV-TW (Taiwan)[1]
CCYV-W-HA1-1 (South Korea)[1]	CCYV-W-JE7 (South Korea)[1]
CCYV-W-UR16 (South Korea)[1]	CCYV-XinJiang (China)[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
Abutilon yellows virus (AbYV)[1]	Crinivirus abutilonis
Bean yellow disorder virus (BnYDV)[1]	Crinivirus flavibetae
Beet pseudoyellows virus (BPYV)	Crinivirus pseudobetae
Beet yellows virus (BYV)	Closterovirus flavibetae
Blackberry yellow vein-associated virus (BYVaV)[1]	Crinivirus rubi
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucurbit yellow stunting disorder virus (CYSDV)	Crinivirus cucurbitae
Diodia vein chlorosis virus (DVCV)[1]	Crinivirus diodiae
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri
Lettuce chlorosis virus (LCV)	Crinivirus lactucachlorosi
Lettuce infectious yellows virus (LIYV)[1]	Crinivirus lactucaflavi
Melon necrotic spot virus (MNSV)	Gammacarmovirus melonis
Papaya ringspot virus (PRSV)	Potyvirus papayanuli
Potato yellow vein virus (PYVV)[1]	Crinivirus flavisolani
Squash mosaic virus (SqMV)	Comovirus cucurbitae
Squash vein yellowing virus (SqVYV)	Ipomovirus cucurbitavenaflavi
Strawberry pallidosis-associated virus (SPaV)	Crinivirus palidofragariae
Sweet potato chlorotic stunt virus (SPCSV)[1]	Crinivirus ipomeae
Tetterwort vein chlorosis virus (TwVCV)[1]	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
Watermelon mosaic virus (WMV)	Potyvirus citrulli
Zucchini green mottle mosaic virus (ZGMMV)	Tobamovirus cucurbitae

[1]Predicted non-detection by *in silico* analysis only

Diagnostic Specificity

True Negatives	67
Correct Diagnoses	67
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 stems	Hemp leaves	Hemp petioles	Hemp stems
Melon leaves	Melon petioles	Melon stems	Pumpkin leaves
Pumpkin petioles	Pumpkin stems	Squash leaves	Squash petioles
Squash stems	Watermelon leaves	Watermelon petioles	Watermelon 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	146
Replicates per Sample	2 - 8
Total Replicates	322
Replicates in Agreement	322
Percent Agreement	100%

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 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.

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