



Test Characteristics

Test Name	Cucumber vein yellowing virus	Test Label	FAM-labeled target probe
Catalog Number	14100	Internal Control	N/A
Acronym	CVYV	Format	XRT
Genus	Ipomovirus	Diluents	GEB2/PD1
Binomial Name	Ipomovirus cucumisvenafavi	Sample Dilution	1:20

Summary

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

Diagnostic Sensitivity

True Positives	121
Correct Diagnoses	119
Percent	98.3%

Analytical Sensitivity

Analytical Sensitivity: The assay is 62.5% 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=16)
 The assay has a 25.0% detection rate at 10 fg/μL with RNA transcripts. (n=16)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CVYV-PV-0724 (Spain)	CVYV-Esp (Spain) ¹
CVYV-ISR (Israel) ¹	CVYV-JOR (Jordan) ¹
CVYV-LIB (Lebanon)	CVYV-OM04-06 (Oman)
CVYV-PV-0776 (Portugal)	CVYV-PV-0997 (Sudan)
CVYV-PV-1291 (Israel)	CVYV-PV-1292 (Israel)
CVYV-SU12-10 (Sudan)	CVYV-SU98-84 (Sudan) ¹
CVYV-TN05-56 (Tunisia) 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
Alfalfa mosaic virus (AMV)	Alfamovirus AMV
Cassava brown streak virus (CBSV) ¹	Ipomovirus brunusmanihotis
Coccinia mottle virus (CocMoV) ¹	Ipomovirus coccinia

Does Not Cross-react With:

Virus Name	Species Name
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucurbit aphid-borne yellows virus (CABYV)	Polerovirus CABYV
Cucurbit yellow stunting disorder virus (CYSDV)	Crinivirus cucurbitae
Kalanchoe mosaic virus (KMV)	Potyvirus kalanchoes
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri
Melon necrotic spot virus (MNSV)	Gammacarmovirus melonis
Papaya ringspot virus (PRSV)	Potyvirus papayanuli
Pepper mild mottle virus (PMMoV) ¹	Tobamovirus capsici
Squash mosaic virus (SqMV)	Comovirus cucurbitae
Squash vein yellowing virus (SqVYV)	Ipomovirus cucurbitavenaflavi
Sweet potato mild mottle virus (SPMMV) ¹	Ipomovirus lenisbatatae
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato mild mottle virus (TMMoV) ¹	Ipomovirus lycopersici
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatussellati
Turnip vein-clearing virus (TVCV)	Tobamovirus rapae
Ugandan cassava brown streak virus (UCBSV) ¹	Ipomovirus manihotis
Watermelon mosaic virus (WMV)	Potyvirus citrulli
Zucchini yellow mosaic virus (ZYMV)	Potyvirus cucurbitaflavitesellati

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

Diagnostic Specificity

True Negatives 58
 Correct Diagnoses 58
 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	Melon leaves	Melon petioles	Melon stems
Pumpkin leaves	Pumpkin petioles	Pumpkin 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	177
Replicates per Sample	2 - 8
Total Replicates	404
Replicates in Agreement	397
Percent Agreement	98.3%

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 specificity³:	(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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