AmplifyRP® XRT for SqVYV
Validation Report
Squash vein yellowing virus
Product No. XCS 91800

Test Characteristics

Test Name	Squash vein yellowing virus	**Test Label**	FAM-labeled target probe
Catalog Number	91800	**Internal Control**	N/A
Acronym	SqVYV	**Format**	XRT
Genus	Ipomovirus	**Diluents**	GEB2/PD1
Binomial Name	Ipomovirus cucurbitavenaflavi	**Sample Dilution**	1:20

Summary

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

Diagnostic Sensitivity

True Positives	135
Correct Diagnoses	133
Percent	98.5%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 62.5% sensitive between 100 ag/µL and 1 fg/µL. (n=8)
Limit of Detection:	The assay has a 100% detection rate at 1 fg/µL with purified virus. (n=4)
	The assay has a 25% detection rate at 100 ag/µL with purified virus. (n=4)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

SqVYV-Baghdad-MS89 (Iraq)	SqVYV-DSMZ PV-1224 (USA)[1]
SqVYV-DSMZ PV-1348 (USA)	SqVYV-Florida (USA)
SqVYV-IL (Israel)[1]	SqVYV-Ir (Iran)[1]
SqVYV-SM2008cHe (FL, USA)[1]	SqVYV-SVYV/Iraq (Iraq)[1]
SqVYV Texas, USA isolate	
[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
Alfalfa mosaic virus (AMV)	Alfamovirus AMV
Algerian watermelon mosaic virus (AWMV)[1]	Potyvirus algeriaense
Cassava brown streak virus (CBSV)[1]	Ipomovirus brunusmanihotis
Coccinia mottle virus (CocMoV)[1]	Ipomovirus cocciniae

Does Not Cross-react With:

Virus Name	Species Name
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucumber vein yellowing virus (CVYV)	Ipomovirus cucumisvenaflavi
Cucumber vein-clearing virus (CuVCV)[1]	Carlavirus cucumis
Cucurbit leaf crumple virus (CuLCrV)[1]	Begomovirus cucurbitae
Melon necrotic spot virus (MNSV)	Gammacarmovirus melonis
Moroccan watermelon mosaic virus (MWMV)[1]	Potyvirus citrullimoroccense
Papaya ringspot virus (PRSV)	Potyvirus papayanuli
Squash mosaic virus (SqMV)	Comovirus cucurbitae
Sweet potato mild mottle virus (SPMMV)[1]	Ipomovirus lenisbatatae
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato leaf curl New Delhi virus (ToLCNDV)	Begomovirus solanumdelhiense
Tomato mild mottle virus (TMMoV)[1]	Ipomovirus lycopersici
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatessellati
Ugandan cassava brown streak virus (UCBSV)[1]	Ipomovirus manihotis
Watermelon leaf mottle virus (WLMV)[1]	Potyvirus citrullufolimaculae
Watermelon mosaic virus (WMV)	Potyvirus citrulli
Zucchini yellow mosaic virus (ZYMV)	Potyvirus cucurbitaflavitesselati
[1]Predicted non-detection by *in silico* analysis only	

Diagnostic Specificity

True Negatives 61
Correct Diagnoses 61
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	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	196
Replicates per Sample	2 - 3
Total Replicates	416
Replicates in Agreement	409
Percent Agreement	98.3%

Reproducibility

Number of Samples	24
Replicates per Sample	3
Number of Operators	4
Total Replicates	288
Replicates in Agreement	287
Percent Agreement	99.7%

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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