AmplifyRP® XRT for BPYV & SPaV
Validation Report
Product No. XCS 87000
Beet pseudoyellows virus and Strawberry pallidosis-associated virus

Test Characteristics

Test Name	Beet pseudoyellows virus and Strawberry pallidosis-associated virus	**Test Label**	FAM-labeled target probe (BPYV) CalRed-labeled target probe (SPaV)
Catalog Number	87000	**Internal Control**	N/A
Acronym	BPYV & SPaV	**Format**	XRT
Genus	Crinivirus	**Diluents**	GEB2/PD1
Binomial Name	Crinivirus pseudobetae (BPYV) Crinivirus palidofragariae (SPaV)	**Sample Dilution**	1:20

Summary

AmplifyRP XRT for BPYV & SPaV is a rapid RNA amplification and detection platform designed for testing cucurbits, ornamentals, strawberries, and other vegetable crops for Beet pseudoyellows virus and Strawberry pallidosis-associated virus. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify and differentiate BPYV & SPaV RNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

BPYV	**True Positives**	159	**SPaV**	**True Positives**	157
	Correct Diagnoses	151		**Correct Diagnoses**	154
	Percent	95.0%		**Percent**	98.1%

Analytical Sensitivity

BPYV	**Analytical Sensitivity:**	The assay is 81.3% sensitive between 100 fg/µL and 300 fg/µL. (n=16)
	Limit of Detection:	The assay has a 100% detection rate at 300 fg/µL with RNA transcripts. (n=8)
		The assay has a 62.5% detection rate at 100 fg/µL with RNA transcripts. (n=8)
SPaV	**Analytical Sensitivity:**	The assay is 68.8% sensitive between 10 fg/µL and 100 fg/µL. (n=16)
	Limit of Detection:	The assay has a 100% detection rate at 100 fg/µL with RNA transcripts. (n=8)
		The assay has a 37.5% detection rate at 10 fg/µL with RNA transcripts. (n=8)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

BPYV-MI (Japan)[1]	SPaV-FJ (China)[1]
SPaV-M1 (MD, USA)	SPaV-SPaV/H2435 (USA)[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 yellows virus (BYV)	Closterovirus flavibetae
Blackberry vein banding-associated virus (BVBaV)[1]	Ampelovirus venarubi
Blackberry yellow vein-associated virus (BYVaV)[1]	Crinivirus rubi
Carrot yellow leaf virus (CYLV)[1]	Closterovirus flavicarotae
Cucurbit chlorotic yellows virus (CCYV)[2]	N/A
Cucurbit yellow stunting disorder virus (CYSDV)	Crinivirus cucurbitae
Diodia vein chlorosis virus (DVCV)[1]	Crinivirus diodiae
Lettuce chlorosis virus (LCV)	Crinivirus lactucachlorosi
Lettuce infectious yellows virus (LIYV)[1]	Crinivirus lactucaflavi
Potato yellow vein virus (PYVV)[1]	Crinivirus flavisolani
Raspberry leaf mottle virus (RLMV)[1]	Closterovirus macularubi
Strawberry chlorotic fleck-associated virus (SCFaV)[1]	Closterovirus fragariae
Sweet potato chlorotic stunt virus (SPCSV)[1]	Crinivirus ipomeae
Tetterwort vein chlorosis virus (TwVCV)[1]	Crinivirus chelidonii
Tomato chlorosis virus (ToCV)	Crinivirus tomatichlorosis
Tomato infectious chlorosis virus (TICV)	Crinivirus contagichlorosis
[1]Predicted non-detection by in silico analysis only	
[2]Described by ICTV as a related, unclassified Crinivirus	

Diagnostic Specificity

	True Negatives	243		**True Negatives**	255
BPYV	**Correct Diagnoses**	243	**SPaV**	**Correct Diagnoses**	255
	Percent	100%		**Percent**	100%

Selectivity:

No Matrix Effect Observed With:			
Beet leaves	Beet petioles	Bitter gourd leaves	Bitter gourd petioles
Black raspberry leaves[1]	Black raspberry petioles	Blackberry leaves	Blackberry petioles
Bottle gourd leaves	Bottle gourd petioles	Cantaloupe leaves	Cantaloupe petioles
Carrot leaves	Carrot petioles	Columbine leaves	Columbine petioles
Cucumber leaves	Cucumber petioles	Cucumber stems	Lettuce leaves
Lettuce petioles	Marigold leaves	Marigold petioles	Melon leaves
Melon petioles	Pumpkin leaves	Pumpkin petioles	Raspberry leaves
Raspberry petioles	Squash leaves	Squash leaves	Squash petioles
Squash petioles	Strawberry leaves	Strawberry petioles	Strawberry rhizome
Strawberry stems	Watermelon leaves	Watermelon petioles	Zinnia leaves
Zinnia petioles			
[1]Inhibition observed.			
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:			
Black raspberry leaves[1]			
[1]Inhibition observed.			

Repeatability

Number of Samples	814
Replicates per Sample	2 - 3
Total Replicates	1146
Replicates in Agreement	1101
Percent Agreement	96.1%

Reproducibility

Number of Samples	48
Replicates per Sample	3
Number of Operators	4
Total Replicates	576
Replicates in Agreement	566
Percent Agreement	98.3%

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 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.

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