



AmplifyRP® XRT for HSVd
Validation Report
Hop stunt viroid
Product No. XCS 64200



Test Characteristics

Test Name	Hop stunt viroid	Test Label	FAM-labeled target probe
Catalog Number	64200	Internal Control	N/A
Acronym	HSVd	Format	XRT
Genus	Hostuviroid	Diluents	GEB/PD1
Binomial Name	Hostuviroid impedihumuli	Sample Dilution	1:20

Summary

AmplifyRP XRT for HSVd is a rapid RNA amplification and detection platform designed for testing of citrus, hemp, hop, and grape for Hop stunt viroid. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify HSVd RNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	68
Correct Diagnoses	68
Percent	100%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 92.5% sensitive between 1 pg/μL and 100 fg/μL. (n=40)
Limit of Detection:	The assay has a 100% detection rate at 1 pg/μL with RNA transcripts. (n=20)
	The assay has a 85.0% detection rate at 100 fg/μL with RNA transcripts. (n=20)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

HSVd citrus-type	HSVd hop-type
HSVd plum-type	HSVd-CAZ2 (Mexico)
HSVd-CC-H	HSVd-cl2
HSVd-E77_HSVd (Spain)	HSVd-HSVd.apr20 (Cyprus)
HSVd-HSVd-maz	HSVd-HSVd-Mor-ValL_Big
HSVd-HT2 (Iran)	HSVd-PM-26x
HSVd-SC-18 (China)	HSVd-tk4 (Turkey)

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
American hop latent virus (AHLV)	Carlavirus americanense
Apple fruit crinkle viroid (AFCVd) ¹	N/A
Apple mosaic virus (ApMV)	Ilarvirus ApMV

Does Not Cross-react With:

Virus Name	Species Name
Arabid mosaic virus (ArMV)	Nepovirus arabis
Dahlia latent viroid (DLVd)	Hostuviroid latensdahliae
Hop latent viroid (HLVd)	Cocadviroid latenshumuli
Hop latent virus (HpLV)	Carlavirus latenshumuli
Hop mosaic virus (HpMV)	Carlavirus humuli
'AFCVd is a tentative member of the genus Apscaviroid	

Diagnostic Specificity

True Negatives 60
Correct Diagnoses 60
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Citrus leaves	Cucumber leaves	Grape leaves	Hemp leaves
Hemp roots	Hop leaves	Hop roots	
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 128
Replicates per Sample 2 - 3
Total Replicates 280
Replicates in Agreement 279
Percent Agreement 99.6%

Reproducibility

Number of Samples 24
Replicates per Sample 3
Number of Operators 4
Total Replicates 288
Replicates in Agreement 280
Percent Agreement 97.2%

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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