



Test Characteristics

Test Name	Blueberry scorch virus	Test Label	FAM-labeled target probe
Catalog Number	19100	Internal Control	N/A
Acronym	BScV	Format	XRT
Genus	Carlavirus	Diluents	GEB/PD1
		Sample Dilution	1:10

Summary

AmplifyRP XRT for BScV is a rapid RNA amplification and detection platform designed for field-based or laboratory testing of blueberries for *Blueberry scorch virus*. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify BScV RNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	100
Correct Diagnoses	100
Percent	100%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 85.0% sensitive between 3 pg/μL and 3 fg/μL. (n=24)
Limit of Detection:	The assay has a 100% detection rate at 30 fg/μL with RNA transcript. (n=10)
	The assay has a 40% detection rate at 3 fg/μL with RNA transcript. (n=10)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

BScV-3 (Canada) ¹	BScV-BC-1 (Canada) ¹
BScV-BC-2 (Canada)	BScV-Cloverdale 1 (Canada) ¹
BScV-Matsqui4 (Canada) ¹	BScV-Nelson (Canada) ¹
BScV-NJ-1 (Sheep Pen Hill Disease) ¹	BScV-NJ-2 (Sheep Pen Hill Disease)
BScV-Rancoca (Canada) ¹	BScV-Sohal (Canada) ¹
BScV-WA-2 ¹	

¹Predicted detection by *in silico* analysis only.

Exclusivity:

Cross-reacts With:

None Known	
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Does Not Cross-react With:

Aconitum latent virus (AcLV) ¹	Amalgavirus vaccinii (Blueberry latent virus) (BBLV) ¹
American hop latent virus (AHLV) ¹	Blueberry shock virus (BShV)
Blueberry shoestring virus (BSSV)	Blueberry virus S (BluVS)
Butterbur mosaic virus (ButMV) ¹	Carnation latent virus (CLV)
Carnation mottle virus (CarMV)	Chrysanthemum virus B (CVB)
Coleus vein necrosis virus (CVNV) ¹	Cowpea mild mottle virus (CPMMV)

Does Not Cross-react With:

Daphne virus S (DVS) ¹	Gaillardia latent virus (GLV) ¹
Garlic common latent virus (GarCLV)	Helleborus net necrosis virus (HNNV) ¹
Hippeastrum latent virus (HiLV) ¹	Hop latent virus (HpLV) ¹
Hop mosaic virus (HpMV) ¹	Hydrangea chlorotic mottle virus (HdCMV) ¹
Kalanchoë latent virus (KLV)	Ligustrum necrotic ringspot virus (LNRSV) ¹
Lily symptomless virus (LSV)	Melon yellowing-associated virus (MYaV) ¹
Mirabilis jalapa mottle virus (MJMV) ¹	Narcissus common latent virus (NCLV) ¹
Nepovirus avii (Cherry leaf roll virus) (CLRV)	Nepovirus lycopersici (Tomato ringspot virus) (ToRSV)
Nepovirus myrtilli (Blueberry leaf mottle virus) (BLMoV)	Nepovirus nicotianae (Tobacco ringspot virus) (TRSV)
Nepovirus persicae (Peach rosette mosaic virus) (PRMV)	Nerine latent virus (NeLV)
Passiflora latent virus (PLV) ¹	Pea streak virus (PeSV) ¹
Phlox virus B (PhIVB) ¹	Phlox virus S (PhIVS) ¹
Poplar mosaic virus (PopMV) ¹	Potato latent virus (PotLV)
Potato virus H (PVH) ¹	Potato virus M (PVM) ¹
Potato virus P (PVP) ¹	Potato virus S (PVS)
Potato virus X (PVX)	Pseudomonas syringae pv.syringae (Pss)
Red clover vein mosaic virus (RCVMV) ¹	Rhizobium rhizogenes ¹
Shallot latent virus (SLV) ¹	Stralarivirus fragariae (Strawberry latent ringspot virus) (SLRSV)
Sweet potato C6 virus (SPC6V) ¹	Sweet potato chlorotic fleck virus (SPCFV) ¹
Tobacco streak virus (TSV)	Xylella fastidiosa (Xf)

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

Diagnostic Specificity

True Negatives 86
Correct Diagnoses 86
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Blueberry leaves	Blueberry petioles	Blueberry stems	Cranberry leaves
Elderberry leaves	Huckleberry leaves	Ligonberry leaves	

Repeatability

Number of Samples 186
Replicates per Sample 2 - 8
Total Replicates 474
Replicates in Agreement 473
Percent Agreement 99.8%

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 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 sensitivity²:** 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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