AmplifyRP® XRT for BN
Validation Report
Phytoplasma solani Bois Noir
Product No. XCS 89000

Test Characteristics

Test Name	Phytoplasma solani Bois Noir	**Test Label**	FAM-labeled target probe
Catalog Number	89000	**Internal Control**	Endogenous
Acronym	BN	**Format**	XRT
Genus	Phytoplasma	**Diluents**	GEB/PD1
Binomial Name	Candidatus Phytoplasma solani	**Sample Dilution**	1:20

Summary

AmplifyRP XRT for BN is a rapid DNA amplification and detection platform designed for testing grapevines for Phytoplasma solani Bois Noir. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify BN DNA and an endogenous DNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	18
Correct Diagnoses	18
Percent	100%

Analytical Sensitivity

Analytical Sensitivity: The assay is 66.7% sensitive between 50 zg/μL and 500 zg/μL. (n=8)

Limit of Detection: The assay has a 100% detection rate at 500 zg/μL with DNA fragment. (n=5)

The assay has a 33.3% detection rate at 50 zg/μL with DNA fragment. (n=3)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

BN-231/09[1]	BN-284/09[1]
BN-Portugal1 (Portugal)[1]	BN-SBR[1]
BN-StC (France)	BN-Stol C[1]
BN-Stol11[1]	BN-Stol11_1_C[1]
BN-Stol11_2_U[1]	BN-Stol11_3_C[1]
BN-Stol11-Conv12/2011-Bg (Bulgaria)[1]	BN-Stol11-Conv2/2010-Bg (Bulgaria)[1]
BN-Stol11-Rubus1/2010-Bg (Bulgaria)[1]	BN-STOL2 (Serbia)[1]
BN-STOL3 (Serbia)[1]	
[1]Predicted detection by *in silico* analysis only	

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Phytoplasma americanum[1]	Phytoplasma asteris[1]
Phytoplasma australiense[1]	Phytoplasma convolvuli[1]
Phytoplasma fragariae[1]	Phytoplasma japonicum[1]
Phytoplasma mali[1]	Phytoplasma pruni[1]
Phytoplasma prunorum[1]	Phytoplasma pyri[1]
Phytoplasma vitis	
[1]Predicted non-detection by *in silico* analysis only	

Diagnostic Specificity

True Negatives	18
Correct Diagnoses	18
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Grape canes	Grape leaves	Grape petioles	

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	7
Replicates per Sample	2
Total Replicates	14
Replicates in Agreement	14
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 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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