AmplifyRP® XRT for *Bcin*
Validation Report
Botrytis cinerea
Product No. XCS 47300

Test Characteristics

Test Name	Botrytis cinerea	**Test Label**	Rox-labeled target probe
Catalog Number	47300	**Internal Control**	N/A
Acronym	Bcin	**Format**	XRT
Genus	Botrytis	**Diluents**	AMP1/PD1
		Sample Dilution	1:20

Summary

AmplifyRP XRT for Bcin is a rapid DNA amplification and detection platform designed for testing grape, hemp (cannabis), rose, and soil for Botrytis cinerea. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify Bcin DNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	115
Correct Diagnoses	110
Percent	95.7%

Analytical Sensitivity

Analytical Sensitivity: The assay is 91.7% sensitive between 1 fg/µL and 10 fg/µL. (n=12)

Limit of Detection: The assay has a 100% detection rate at 10 fg/µL with DNA fragment. (n=6)

The assay has a 83.3% detection rate at 1 fg/µL with DNA fragment. (n=6)

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of Bcin. One hundred ten distinct samples of Bcin have been experimentally proven to be detected including Botrytis cinerea (asexual stage) and Botrytis fuckeliana (sexual stage).

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Botrytis aclada[1]	Botrytis allii[1]
Botrytis arisaemae[1]	Botrytis byssoidea[1]
Botrytis californica[1]	Botrytis caroliniana[1]
Botrytis convoluta[1]	Botrytis croci[1]
Botrytis cryptomeriae[1]	Botrytis deweyae[1]
Botrytis elliptica[1]	Botrytis eucalypti[1]
Botrytis euroamericana[1]	Botrytis fabae[1]
Botrytis fabiopsis[1]	Botrytis fragariae[1]
Botrytis galanthina[1]	Botrytis hyacinthi[1]
Botrytis macadamiae[1]	Botrytis mali[1]
Botrytis medusae[1]	Botrytis paeoniae[1]
Botrytis pelargonii[1]	Botrytis porri[1]
Botrytis prunorum[1]	Botrytis pseudocinerea[1]
Botrytis pyriformis[1]	Botrytis sinoallii[1]
Botrytis sinoviticola[1]	Botrytis squamosa[1]

Does Not Cross-react With:

Botrytis tulipae[1]	Cladosporium westeerdijkieae[1]
Colletotrichum acutatum	Colletotrichum fioriniae
Fusarium oxysporum	Fusarium proliferatum
Fusarium solani	Fusarium sporotrichioides
Fusarium verticilliodes[1]	Golovinomyces ambrosiae[1]
Golovinomyces cichoracearum[1]	Golovinomyces spadiceus[1]
Penicillium olsonii	Pythium aphanidermatum[1]
Pythium dissotocum[1]	Pythium myriotylum[1]
Rhizopus stolonifer[1]	Sclerotinia sclerotiorum
[1]Predicted non-detection by in silico analysis only.	

Diagnostic Specificity

True Negatives	52
Correct Diagnoses	52
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Bean leaves	Cannabis (Hemp) buds	Cannabis (Hemp) leaves	Cannabis (Hemp) stems
Cucumber leaves	Grape fruit	Grape stems	Pepper fruit
Pepper leaves	Rose flowers	Rose leaves	Soil
Strawberry calyx	Strawberry fruit	Strawberry leaves	Tomato calyx
Tomato fruit	Tomato leaves	Water	

Repeatability

Number of Samples	167
Replicates per Sample	2 - 6
Total Replicates	363
Replicates in Agreement	348
Percent Agreement	95.9%

Reproducibility

Number of Samples	24
Replicates per Sample	3
Number of Operators	4
Total Replicates	288
Replicates in Agreement	282
Percent Agreement	97.9%

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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