AmplifyRP® XRT for Lso
Validation Report
Candidatus Liberibacter solanacearum
Product No. XCS 66100

Test Characteristics

Test Name	Candidatus Liberibacter solanacearum	**Test Label**	FAM-labeled target probe
Catalog Number	66100	**Internal Control**	N/A
Acronym	Lso	**Format**	XRT
Genus	Liberibacter	**Diluents**	AMP1/PD1
		Sample Dilution	1:20

Summary

AmplifyRP® XRT for Lso is a rapid DNA amplification and detection platform designed for testing potatoes for Candidatus Liberibacter solanacearum. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify Lso DNA at a single operating temperature (39 °C).

Diagnostic Sensitivity

True Positives	116
Correct Diagnoses	120
Percent	96.7%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 68.8% sensitive between 10 ag/µL and 100 ag/µL. (n=16)
Limit of Detection:	The assay has a 100% detection rate at 100 ag/µL with DNA fragment. (n=8)
	The assay has a 37.5% detection rate at 10 ag/µL with DNA fragment. (n=8)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

Lso haplotype A (solanaceous) (USA)[1]	Lso haplotype B (solanaceous) (El Salvador)
Lso haplotype C (carrot/celery) (Germany)[1]	Lso haplotype D (carrot/celery) (Greece)
Lso haplotype E	Lso haplotype F (solanaceous) (USA)[1]
[1]Based on *in silico* analysis	

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Agrobacterium tumefaciens	Clavibacter michiganensis subsp. michiganensis (Cmm)
Clavibacter michiganensis subsp. sepedonicus (Cms)	Dickeya dianthicola[1]
Dickeya solani[1]	Liberibacter asiaticus
Liberibacter brunswickensis[1]	Phytoplasma asteris[1]
Phytoplasma aurantifolia[1]	Phytoplasma trifolii[1]
Ralstonia solanacearum (Rs)	
[1]Based on *in silico* analysis	

Diagnostic Specificity

True Negatives	65
Correct Diagnoses	65
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Carrot midribs	Carrot petioles	Carrot stems	Eggplant midribs
Eggplant petioles	Eggplant stems	Pepper midribs[1]	Pepper petioles[1]
Pepper stems[1]	Potato midribs	Potato petioles	Potato stems
Potato tubers	Tomato midribs	Tomato petioles	Tomato stems
[1]100-fold decrease in sensitivity 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:			
None Known			

Repeatability

Number of Samples	185
Replicates per Sample	2 - 8
Total Replicates	446
Replicates in Agreement	429
Percent Agreement	96.2%

Reproducibility

Number of Samples	24
Replicates per Sample	3
Number of Operators	4
Total Replicates	288
Replicates in Agreement	284
Percent Agreement	98.6%

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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