AmplifyRP® XRT for *Rs*
Validation Report
Ralstonia solanacearum
Product No. XCS 33900

Test Characteristics

Test Name	Ralstonia solanacearum	**Test Label**	FAM-labeled target probe
Catalog Number	33900	**Internal Control**	N/A
Acronym	Rs	**Format**	XRT
Genus	Ralstonia	**Diluents**	AMP1/PD1
Binomial Name	Ralstonia solanacearum	**Sample Dilution**	1:20

Summary

AmplifyRP® XRT for Rs is a rapid DNA amplification and detection platform designed for field-based or laboratory testing of Ralstonia solanacearum in many crops including: banana, eggplant, ginger, pepper, potato, tomato, geranium and many other ornamental species. Note: Analytical sensitivity when testing pepper tissue is approximately 1,000-fold lower than with other hosts. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify Rs DNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	128
Correct Diagnoses	127
Percent	99.2%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 75% sensitive between 100 fg/µL and 10 fg/µL. (n=16)
Limit of Detection:	The assay has a 100% detection rate at 100 fg/µL with DNA fragments. (n=8)
	The assay has a 50% detection rate at 10 fg/µL with DNA fragments. (n=8)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

Rs, race 1	Rs, race 2
Rs, race 3	Rs, race 3 biovar 2
Rs, race 4	Rs, race 5
Rs-12-4-5 WI (WI, USA)	Rs-12-4-6 SD (NY, USA)
Rs-22056-99 (USA)	Rs-399CP (A4087) (USA)
Rs-3R (A4654) (HI, USA)[1]	Rs-4B-1 (A4646) (USA)[1]
Rs-4B-3 (A4647) (USA)[1]	Rs-4B-4 (A4648) (USA)[1]
Rs-4B-8 (A4651) (USA)[1]	Rs-4B-9 (A4652) (USA)[1]
Rs-6A-1 (A4635) (Indonesia)	Rs-909-87 (A3910) (USA)[1]
Rs-A3294 (USA)	Rs-A3381 (Costa Rica)
Rs-A3447 (Columbia)[1]	Rs-A3450 (Trinidad and Tobago)[1]
Rs-A3903 (HI, USA)[1]	Rs-A476-1B (A3344) (USA)[1]
Rs-B185 (A5151) (HI, USA)[1]	Rs-BB-Feutz (WI, USA)[1]
Rs-BD-1 (A4606) (Indonesia)[1]	Rs-Bd-3 (A4609) (Indonesia)
Rs-BD-6 (A4612) (Indonesia)	Rs-BIOTE1690 (A4128) (Philippines)
Rs-BIOTE1694 (A4125) (Philippines)[1]	Rs-BIOTE1705 (A4126) (Philippines)
Rs-C1016A (A3914) (HI, USA)[1]	Rs-C1017-1A (A3911) (USA)[1]
Rs-CFBP 8695 (Iran)[1]	Rs-CFPB 8697 (Iran)[1]
Rs-DP-11 (A5183) (USA)[1]	Rs-DP-13 (A5184) (USA)[1]

Isolates and Geographic Regions Detected:

Rs-DP-22 (A5185) (USA)[1]	Rs-DP-6 (A5181) (USA)[1]
Rs-DP-9 (A5182) (USA)	Rs-GMI1000 (French Guyana)
Rs-GW-1 (A4515) (USA)[1]	Rs-GW-2 (A4516) (USA)[1]
Rs-IPO1609 (Philippines)	Rs-K60 (NC, USA)[1]
Rs-KV1.1 (A3906) (USA)[1]	Rs-KV1.2 (A3907) (USA)[1]
Rs-KV2.2 (A3908) (USA)	Rs-KV3.1 (A3909) (USA)[1]
Rs-KV5.1 (A3913) (USA)[1]	Rs-MI20 (MI, USA)
Rs-Molk2 (Philippines)[1]	Rs-P5 (A3904) (USA)[1]
Rs-PS1 (India)	Rs-PS-11 (A4613) (Guatemala)[1]
Rs-PS7 (A3915) (HI, USA)[1]	Rs-QR87 (A5156) (USA)
Rs-S-118 (A4492) (Costa Rica)[1]	Rs-S-164 (A4493) (Costa Rica)[1]
Rs-S-200 (A4494) (Honduras)[1]	Rs-S215 (A4495) (Venezuela)[1]
Rs-T6 (A3776) (Philippines)[1]	Rs-UW119 (Costa Rica)
Rs-UW130 (A3459) (Peru)[1]	Rs-UW152 (Australia)
Rs-UW170 (A4497) (Colombia)[1]	Rs-UW256 (Costa Rica)
Rs-UW276 (Mexico)[1]	Rs-UW35 (A4496) (Colombia)
Rs-UW359 (China)	Rs-UW360 (China)
Rs-UW365 (China)	Rs-UW373 (China)
Rs-UW464 (Brazil)	Rs-UW551 (Kenya)
Rs-UW602 (Guatemala)	Rs-UW656 (Brazil)
Rs-UW700 (VA, USA)	Rs-UW757 (Guatemala)
[1]Predicted detection by *in silico* analysis only.	

Exclusivity:

Cross-reacts With:

None Known	

Does Not Cross-react With:

Acidovorax avenae	Acidovorax avenae subsp. citrulli
Acinetoacter calcoaceticus[1]	Agrobacterium tumefaciens
Agrobacterium vitis	Bacillus cereus[1]
Bacillus subtilis[1]	Bacillus thuringiensis
Burkholderia glumae	Clavibacter michiganensis subsp. insidiosus
Clavibacter michiganensis subsp. michiganensis	Clavibacter michiganensis subsp. nebraskensis
Clavibacter michiganensis subsp. sepedonicus	Clavibacter michiganensis subsp. tessellaris
Cupriavidus basilensis	Cupriavidus necator
Curtobacterium flaccumfaciens pv. poinsettia	Dickeya chrysanthemi
Erwinia amylavora	Erwinia tracheiphilia
Escherichia coli	Pantoae agglomerans
Pantoea ananatis	Pantoea stewartii
Pectobacterium atrosepticum	Pectobacterium caraotovarum
Pseudomonas aeruginosa	Pseudomonas chichorii
Pseudomonas fluorescens	Pseudomonas syringae pv. glycinea
Pseudomonas syringae pv. porri	Pseudomonas syringae pv. syringae
Pseudomonas syringae pv. tomato	Pseudomonas syringeae pv. phaseolicola
Ralstonia insidiosa	Rhizobium radiobacter

Does Not Cross-react With:

Rhizobium rhizogenes	Rhodococcus fascians
Serratia marcescens[1]	Sphingomonas spp.
Spiroplasma citri[1]	Staphylococcus aureus[1]
Stenotrophomonas maltophilia	Streptococcus faecalis[1]
Xanthomonas albilineans	Xanthomonas arboricola pv. pruni
Xanthomonas axonopodis pv. begoniae	Xanthomonas axonopodis pv. citri
Xanthomonas axonopodis pv. dieffenbachiae	Xanthomonas axonopodis pv. phaseoli
Xanthomonas campestris pv. armoracieae	Xanthomonas campestris pv. campestris
Xanthomonas campestris pv. oryzae[1]	Xanthomonas campestris pv. zinniae
Xanthomonas citri pv. aurantifolii	Xanthomonas citri pv. malvacearum
Xanthomonas citrumelo	Xanthomonas euvesicatoria
Xanthomonas fragariae	Xanthomonas gardneri
Xanthomonas hortorum pv. pelargonii	Xanthomonas perforans
Xanthomonas translucens pv. translucens	Xanthomonas vasicola pv. vasculorum
Xanthomonas vesicatoria	Xylella fastidiosa
[1]Predicted non-detection by *in silico* analysis only.	

Diagnostic Specificity

True Negatives 63
Correct Diagnoses 63
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Alocasia leaves	Alocasia petioles	Banana petioles	Banana stems
Eggplant petioles	Eggplant stems	Geranium petioles	Geranium stems
Ginger petioles	Ginger stems	Pepper petioles[1]	Pepper stems[1]
Philodendron leaves	Philodendron petioles	Potato petioles	Potato stems
Rose petioles	Rose stems	Soil	Tomato petioles
Tomato stems	Water (irrigation)		
[1]Analytical sensitivity is approximatley 1,000-fold lower than with other hosts.			
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:			
Pepper petioles[1]	Pepper stems[1]		
[1]Analytical sensitivity is approximatley 1,000-fold lower than with other hosts.			

Repeatability

Number of Samples	191
Replicates per Sample	2 - 4
Total Replicates	410
Replicates in Agreement	405
Percent Agreement	98.8%

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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