



AmplifyRP® XRT for ToFBV

Validation Report

Tomato fruit blotch virus

Product No. XCS 56200



Test Characteristics

Test Name	Tomato fruit blotch virus	Test Label	FAM-labeled target probe
Catalog Number	56200	Internal Control	ROX-labeled control probe (endogenous)
Acronym	ToFBV	Format	XRT
Genus	Blunervirus	Diluents	GEB/PD1
Binomial Name	Blunervirus solani	Sample Dilution	1:20

Summary

AmplifyRP XRT for ToFBV is a rapid RNA amplification and detection platform designed for testing solanaceous crops for Tomato fruit blotch virus. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify ToFBV RNA and an endogenous RNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	125
Correct Diagnoses	120
Percent	96.0%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 77.8% sensitive between 100 ag/μL and 1 fg/μL. (n=18)
Limit of Detection:	The assay has a 100% detection rate at 1 fg/μL with RNA transcripts. (n=9)
	The assay has a 55.6% detection rate at 100 ag/μL with RNA transcripts. (n=9)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

ToFBV Italian isolate 1	ToFBV Italian isolate 2
ToFBV Italian isolate 3	ToFBV Italian isolate 4
ToFBV-2-56 (Australia) ¹	ToFBV-BOL19ST (Slovenia) ¹
ToFBV-BOL19SW (Slovenia) ¹	ToFBV-F02-04-CH (Switzerland) ¹
ToFBV-Fondi2018 (Italy) ¹	ToFBV-GBVC01 (Belgium) ¹
ToFBV-MAL (Brazil) ¹	ToFBV-TFBV/Spanish (Spain) ¹
ToFBV-ToFBV_Tn92 (Tunisia) ¹	ToFBV-ToFBV-Gr (Greece) ¹
¹ Predicted detection by <i>in silico</i> analysis only	

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
Alfalfa mosaic virus (AMV)	Alfavirus AMV
Beet curly top virus (BCTV)	Curtovirus betae
Blueberry necrotic ring blotch virus (BNRBV) ¹	Blunervirus vaccinii

Does Not Cross-react With:

Virus Name	Species Name
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Impatiens necrotic spot virus (INSV)	Orthotospovirus impatiensnecromaculae
Pepino mosaic virus (PepMV)	Potexvirus pepini
Tea plant necrotic ring blotch virus (TPNRBV) ¹	Blunervirus camelliae
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tobacco ringspot virus (TRSV)	Nepovirus nicotianae
Tomato brown rugose fruit virus (ToBRFV)	Tobamovirus fructirugosum
Tomato bushy stunt virus (TBSV)	Tombusvirus lycopersici
Tomato leaf curl new delhi virus (ToLCNDV)	Begomovirus solanumdelhiense
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatusellati
Tomato mottle virus (ToMoV)	Begomovirus solanumvariati
Tomato ringspot virus (ToRSV)	Nepovirus lycopersici
Tomato spotted wilt virus (TSWV)	Orthotospovirus tomatomaculae
¹ Predicted non-detection by <i>in silico</i> analysis only	

Diagnostic Specificity

True Negatives 96
Correct Diagnoses 94
Percent 97.9%

Selectivity:**No Matrix Effect Observed With:**

Eggplant leaves ^{1,2}	Eggplant petioles ^{1,2}	Eggplant roots	Pepper fruit ^{1,2}
Pepper leaves ^{1,2}	Pepper petioles ^{1,2}	Pepper roots	Potato leaves
Potato petioles	Tobacco leaves	Tobacco petioles	Tobacco roots
Tomato fruit ¹	Tomato leaves	Tomato petioles ¹	Tomato roots
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.).			
¹ Inhibition was observed.			
² Utilizing the Inhibitory Tissue Boil Step in the user guide eliminated the inhibition.			

Matrix Effect Observed With:

Eggplant leaves ^{1,2}	Eggplant petioles ^{1,2}	Pepper fruit ^{1,2}	Pepper leaves ^{1,2}
Pepper petioles ^{1,2}	Tomato fruit ¹	Tomato petioles ¹	
¹ Inhibition was observed.			
² Utilizing the Inhibitory Tissue Boil Step in the user guide eliminated the inhibition.			

Repeatability

Number of Samples	220
Replicates per Sample	2 - 8
Total Replicates	486
Replicates in Agreement	461
Percent Agreement	94.9%

Reproducibility

Number of Samples	32
Replicates per Sample	3
Number of Operators	4
Total Replicates	384
Replicates in Agreement	380
Percent Agreement	99.0%

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 sensitivity³:	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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