



AmplifyRP® XRT for ToBRFV
Validation Report
Tomato brown rugose fruit virus
Product No. XCS 66800



Test Characteristics

Test Name	Tomato brown rugose fruit virus	Test Label	FAM-labeled target probe
Catalog Number	66800	Internal Control	ROX-labeled control probe (endogenous)
Acronym	ToBRFV	Format	XRT
Genus	Tobamovirus	Diluents	GEB/PD1
Binomial Name	Tobamovirus fructirugosum	Sample Dilution	1:20

Summary

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

Diagnostic Sensitivity

True Positives	55
Correct Diagnoses	55
Percent	100%

Analytical Sensitivity

Limit of Detection:	Approximately 15 fg/μL of RNA transcripts
Limit of Detection:	1:10,000,000 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

ToBRFV Israel isolate	ToBRFV Italy isolate
ToBRFV Mexico isolate	ToBRFV PV-1300 (Cyprus)
ToBRFV USA isolate	ToBRFV-Tom1-Jo (Jordan)

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None known	

Does Not Cross-react With:

Virus Name	Species Name
African eggplant-associated virus (AEaV) ²	N/A
Bell pepper mottle virus (BPeMV)	Tobamovirus maculacapsici
Brugmansia latent virus (BrLV) ³	N/A
Chili pepper mild mottle virus (CPMMoV) ⁴	N/A
Cucumber green mottle mosaic virus (CGMMV)	Tobamovirus viridimaculae
Cucumber mottle virus (CMoV)	Tobamovirus cucumeris
Frangipani mosaic virus (FrMV)	Tobamovirus frangipani
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri
Maracuja mosaic virus (MarMV)	Tobamovirus maracujae

Does Not Cross-react With:

Virus Name	Species Name
Obuda pepper virus (ObPV)	Tobamovirus obudae
Odontoglossum ringspot virus (ORSV)	Tobamovirus odontoglossi
Paprika mild mottle virus (PaMMV)	Tobamovirus paprikae
Pepper mild mottle virus (PMMoV)	Tobamovirus capsici
Piper chlorosis virus (PChV) ¹	N/A
Rehmannia mosaic virus (ReMV)	Tobamovirus rehmanniae
Ribgrass mosaic virus (RMV)	Tobamovirus plantagonis
Scopolia mild mottle virus (SMMoV) ⁵	N/A
Streptocarpus flower break virus (SFBV)	Tobamovirus streptocarpi
Sunn-hemp mosaic virus (SHMV)	Tobamovirus crotalariae
Tobacco mild green mosaic virus (TMGMV)	Tobamovirus mititessellati
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato mosaic virus (ToMV)	Tobamovirus tomatotessellati
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculotessellati
Turnip vein-clearing virus (TVCV)	Tobamovirus rapae
Wasabi mottle virus (WMoV)	Tobamovirus wasabi
Youcai mosaic virus (YoMV)	Tobamovirus youcai
Zucchini green mottle mosaic virus (ZGMMV)	Tobamovirus cucurbitae
¹ Piper chlorosis virus (PChV) has been reported to be a possible novel Tobamovirus.	
² African eggplant-associated virus (AEaV) has been reported to be a possible novel Tobamovirus.	
³ Brugmansia latent virus (BrLV) has been reported to be a possible novel Tobamovirus.	
⁴ Chili pepper mild mottle virus (CPMMoV) has been reported to be a possible novel Tobamovirus.	
⁵ Scopolia mild mottle virus (SMMoV) has been reported to be a possible novel Tobamovirus.	

Diagnostic Specificity

True Negatives 59
Correct Diagnoses 59
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Pepper leaves ¹	Pepper seeds ¹	Petunia leaves	Petunia stems
Tobacco leaves	Tobacco stems	Tomato fruit	Tomato leaves
Tomato seeds			
¹ Some pepper varieties did not amplify the internal control. An alternative AmpliFire® QR code for pepper tissue is provided in the user guide. Detection of ToBRFV was not impacted.			
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.).			

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Pepper leaves ¹	Pepper seeds ¹		
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Repeatability

Number of Samples 28
Replicates per Sample 1 - 3
Average Percent Agreement Between Replicates 100%

Reproducibility

Number of Samples 28
Replicates per Sample 1 - 3
Number of Operators 3
Average Percent Agreement Between Replicates Between Operators 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 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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