AmplifyRP® XRT for CABYV
Validation Report
Cucurbit aphid-borne yellows virus
Product No. XCS 51600

Test Characteristics

Test Name	Cucurbit aphid-borne yellows virus	**Test Label**	FAM-labeled target probe
Catalog Number	51600	**Internal Control**	N/A
Acronym	CABYV	**Format**	XRT
Genus	Polerovirus	**Diluents**	GEB5/PD1
Binomial Name	Polerovirus CABYV	**Sample Dilution**	1:20

Summary

AmplifyRP XRT for CABYV is a rapid RNA amplification and detection platform designed for testing cucurbits and other hosts for Cucurbit aphid-borne yellows virus. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify CABYV RNA at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	111
Correct Diagnoses	111
Percent	100%

Analytical Sensitivity

Analytical Sensitivity: The assay is 85% sensitive between 1 fg/µL and 100 ag/µL. (n=20)

Limit of Detection: The assay has a 100% detection rate at 1 fg/µL with RNA transcripts. (n=10)

The assay has a 70% detection rate at 100 ag/µL with RNA transcripts. (n=10)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CABYV (EU000535) (China)	CABYV (EU636992) (China)
CABYV PV-1017 (France)	CABYV-1.1M/M (Spain)[1]
CABYV-10PN (Papua New Guinea)	CABYV-59.1M/A (Spain)
CABYV-C-TW20 (Taiwan)[1]	CABYV-CY3 (South Korea)[1]
CABYV-CY4 (South Korea)[1]	CABYV-CY6 (South Korea)[1]
CABYV-CZ (China)	CABYV-EM160093 (France)[1]
CABYV-FJ (China)	CABYV-GM16 (South Korea)[1]
CABYV-GM7 (South Korea)[1]	CABYV-GS1 (South Korea)[1]
CABYV-GS2 (South Korea)[1]	CABYV-GS6 (South Korea)[1]
CABYV-Hainan (China)	CABYV-HD1 (South Korea)[1]
CABYV-HD118 (South Korea)[1]	CABYV-HS1 (South Korea)[1]
CABYV-HS2 (South Korea)[1]	CABYV-JAN (Japan)[1]
CABYV-LP63 (Spain)	CABYV-N[1]
CABYV-NW1 (South Korea)[1]	CABYV-NW18 (South Korea)[1]
CABYV-NW2 (South Korea)[1]	CABYV-NW2(14) (South Korea)[1]
CABYV-NW5 (South Korea)[1]	CABYV-PF-564 (Brazil)
CABYV-PF-799 (Brazil)	CABYV-PF-M2BA (Brazil)[1]
CABYV-POL-SQ (India)	CABYV-R-TW82 (Taiwan)
CABYV-Sq/2003/7.2 (Spain)[1]	CABYV-Sq/2004/1.9 (Spain)
CABYV-Sq/2005/9.2 (Spain)	CABYV-SW1 (South Korea)[1]

Isolates and Geographic Regions Detected:

CABYV-SW1(14) (South Korea)[1]	CABYV-SW2 (South Korea)[1]
CABYV-SW25 (South Korea)[1]	CABYV-SW64 (South Korea)[1]
[1]Based on *in silico* analysis	

Exclusivity:

Cross-reacts With:

Polerovirus MABYV (Melon aphid-borne yellows virus) (MABYV)[1]	
[1]Non-amplification, hydridization cross-reaction only observed with samples higher than 3.8 x 10^8 target copies.	

Does Not Cross-react With:

Alfamovirus AMV (Alfalfa mosaic virus) (AMV)	Begomovirus solanumdelhiense (Tomato leaf curl New Delhi virus) (ToLCNDV)
Comovirus cucurbitae (Squash mosaic virus) (SqMV)	Cucumovirus CMV (Cucumber mosaic virus) (CMV)
Gammacarmovirus melonis (Melon necrotic spot virus) (MNSV)	Ipomovirus cucumisvenaflavi (Cucumber vein yellowing virus) (CVYV)
Luteovirus phaseoli (Bean leafroll virus) (BLRV)[1]	Orthotospovirus citrullomaculosi (Watermelon silver mottle virus) (WSMoV)
Polerovirus BCHV (Beet chlorosis virus) (BChV)[1]	Polerovirus BMYV (Beet mild yellowing virus) (BMYV)[1]
Polerovirus BWYV (Beet western yellows virus) (BWYV)	Polerovirus CLDV (Cotton leafroll dwarf virus) (CLRDV)[1]
Polerovirus CPCSV (Chickpea chlorotic stunt virus) (CpCSV)[1]	Polerovirus CTRLV (Carrot red leaf virus) (CtRLV)[1]
Polerovirus CYDVRPS (Cereal yellow dwarf virus-RPS) (CYDV-RPS)	Polerovirus CYDVRPV (Cereal yellow dwarf virus-RPV) (CYDV-RPV)
Polerovirus MYDVRMV (Maize yellow dwarf virus-RMV) (MYDV-RMV)	Polerovirus PEVYV1 (Pepper vein yellows virus 1) (PeVYV1)[1]
Polerovirus PEVYV2 (Pepper vein yellows virus 2) (PeVYV2)[1]	Polerovirus PEVYV3 (Pepper vein yellows virus 3) (PeVYV3)[1]
Polerovirus PEVYV4 (Pepper vein yellows virus 4) (PeVYV4)[1]	Polerovirus PEVYV5 (Pepper vein yellows virus 5) (PeVYV5)[1]
Polerovirus PEVYV6 (Pepper vein yellows virus 6) (PeVYV6)[1]	Polerovirus PLRV (Potato leafroll virus) (PLRV)
Polerovirus SABYV (Suakwa aphid-borne yellows virus) (SABYV)[1]	Polerovirus SCYLV (Sugarcane yellow leaf virus) (SCYLV)
Polerovirus TUYV (Turnip yellows virus) (TuYV)	Polerovirus TVDV (Tobacco vein distorting virus) (TVDV)[1]
Potyvirus algeriaense (Watermelon mosaic virus) (WMV)	Potyvirus cucurbitaflavitesselati (Zucchini yellow mosaic virus) (ZYMV)
Potyvirus papayanuli (Papaya ringspot virus) (PRSV)	Tobamovirus capsici (Pepper mild mottle virus) (PMMoV)
Tobamovirus cucurbitae (Zucchini green mottle mosaic virus) (ZGMMV)	Tobamovirus frangipani (Frangipani mosaic virus) (FrMV)
Tobamovirus fructirugosum (Tomato brown rugose fruit virus) (ToBRFV)	Tobamovirus kyuri (Kyuri green mottle mosaic virus) (KGMMV)
Tobamovirus maculatessellati (Tomato mottle mosaic virus) (ToMMV)	Tobamovirus mititessellati (Tobacco mild green mosaic virus) (TMGMV)
Tobamovirus obudae (Obuda pepper virus) (ObPV)	Tobamovirus odontoglossi (Odontoglossum ringspot virus) (ORSV)
Tobamovirus paprikae (Paprika mild mottle virus) (PaMMV)	Tobamovirus plantagonis (Ribgrass mosaic virus) (RMV)
Tobamovirus rapae (Turnip vein-clearing virus) (TVCV)	Tobamovirus rehmanniae (Rehmannia mosaic virus) (ReMV)
Tobamovirus streptocarpi (Streptocarpus flower break virus) (SFBV)	Tobamovirus tabaci (Tobacco mosaic virus) (TMV)
Tobamovirus tomatotessellati (Tomato mosaic virus) (ToMV)	Tobamovirus viridimaculae (Cucumber green mottle mosaic virus) (CGMMV)
Tobamovirus youcai (Youcai mosaic virus) (YoMV)	Tombusvirus cucumis (Cucumber necrosis virus) (CNV)
[1]Based on *in silico* analysis	

Diagnostic Specificity

True Negatives 54
Correct Diagnoses 54
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Bitter gourd leaves	Bitter gourd petioles	Bottle gourd leaves	Bottle gourd petioles
Cucumber leaves	Cucumber petioles	Cucumber stems	Faba bean leaves
Faba bean petioles	Lettuce leaves	Melon leaves	Melon petioles
Melon stems	Pumpkin leaves	Pumpkin petioles	Pumpkin stems
Squash leaves	Squash petioles	Toamto leaves	Toamto petioles
Toamto stems	Watermelon leaves	Watermelon petioles	Watermelon stems
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 136
Replicates per Sample 2 - 8
Total Replicates 306
Replicates in Agreement 305
Percent Agreement 99.7%

Reproducibility

Number of Samples 24
Replicates per Sample 3
Number of Operators 4
Total Replicates 288
Replicates in Agreement 287
Percent Agreement 99.7%

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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