Validation Report: ELISA

SRA 54301 • *Odontoglossum ringspot virus* (ORSV)

Test Characteristics

Test Name	Odontoglossum ringspot virus	**Capture Antibody**	Polyclonal (Rabbit)
Catalog Number	54301	**Detection Antibody**	Polyclonal (Rabbit)
Acronym	ORSV	**Format**	DAS-ELISA
Genus	Tobamovirus	**Diluents**	GEB/RUB6
Binomial Name	Tobamovirus odontoglossi	**Sample Dilution**	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Odontoglossum ringspot virus (ORSV) in orchid leaves. ORSV is a member of the Tobamovirus genus known for their rod-shaped virus particles.

Diagnostic Sensitivity

True Positives	10
Correct Diagnoses	10
Percent	100%

Analytical Sensitivity

Limit of Detection: 1:64,800 dilution of infected tissue (pathogen titer unknown)

Analytical Specificity

Inclusivity:

This assay was designed to detect all strains and isolates of ORSV. Ten distinct samples of ORSV have been experimentally proven to be detected.

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri

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 mosaic virus (CMV)	Cucumovirus CMV
Cucumber mottle virus (CMoV)	Tobamovirus cucumeris
Cymbidium mosaic virus (CymMV)	Potexvirus cymbidii

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Does Not Cross-react With:

Virus Name	Species Name
Cymbidium ringspot virus (CymRSV)	Tombusvirus cymbidii
Frangipani mosaic virus (FrMV)	Tobamovirus frangipani
Maracuja mosaic virus (MarMV)	Tobamovirus maracujae
Obuda pepper virus (ObPV)	Tobamovirus obudae
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 brown rugose fruit virus (ToBRFV)	Tobamovirus fructirugosum
Tomato mosaic virus (ToMV)	Tobamovirus tomatotessellati
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatessellati
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.

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Diagnostic Specificity

True Negatives	51
Correct Diagnoses	51
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Anthurium leaves	Cattleya leaves	Cymbidium leaves	Epidendendrum leaves
Laelia leaves	Phalaenopsis leaves	Spathiphyllum leaves	Spathoglottis leaves
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			

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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