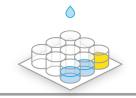
Validation Report: ELISA

PSA/SRA 32100 • Beet western yellows virus (BWYV)



Test Characteristics

Test Name	Beet western yellows virus	Capture Antibody	Polyclonal (Rabbit)
Catalog Number	32100	Detection Antibody	Monoclonal (Mouse)
Acronym	BWYV	Format	Compound-ELISA
Genus	Polerovirus	Diluents	GEB/ECI
		Sample Dilution	1:10

Summary

This ELISA test is a qualitative serological assay for the detection of Beet western yellows virus (BWYV) in ornamental and vegetable leaves. BWYV is a member of the Polerovirus genus known for their Non-enveloped, spherical-shaped virus particles.

Diagnostic Sensitivity		Analytical Sensit	Analytical Sensitivity		
True Positives	80	Limit of Detection:	1:5,000 dilution of infected tissue (pathogen titer unknown)		
Correct Diagnoses	80				
Percent	100%				

Analytical Specificity

Inclusivity:

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

Exclusivity:

Cross-reacts With:

Beet chlorosis virus (BChV)	Beet mild yellowing virus (BMYV)
Cereal yellow dwarf virus RPS (CYDV-RPS)	Cereal yellow dwarf virus RPV (CYDV-RPV)

Does Not Cross-react With:

Alfalfa mosaic virus (AMV)	Barley yellow dwarf virus SGV (BYDV-SGV)
Cucumber mosaic virus (CMV)	Cucurbit aphid-borne yellows virus (CAbYV)
Lettuce mosaic virus (LMV)	Maize yellow dwarf virus RMV (MYDV-RMV)
Potato leafroll virus (PLRV)	Tomato spotted wilt virus (TSWV)
Tombusvirus lycopersici (Tomato bushy stunt virus) (TBSV)	Turnip mosaic virus (TuMV)
Turnip yellows virus (TuYV)	



Diagnostic Specificity

True Negatives 165 Correct Diagnoses 165 Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Alfalfa leaves	Basil leaves	Bean leaves	Beet leaves
Bell pepper leaves	Brassicas leaves	Cannabis (Hemp) leaves	Canola leaves
Clover leaves	Cotton leaves	Cucumber leaves	Eggplant leaves
Geranium leaves	Leek leaves	Lettuce leaves	Onion leaves
Pea leaves	Potato leaves	Radish leaves	Spinach leaves
Squash leaves	Tobacco leaves	Tomato leaves	Turnip leaves
Wheat leaves	Zinnia leaves		

Reproducibility

Repeatability

Number of Samples	245	Number of Samples	27
Replicates per Sample	3 - 18	Replicates per Sample	6
Total Replicates	1718	Number of Operators	3
Replicates in Agreement	1716	Total Replicates	486
Percent Agreement	99.9%	Replicates in Agreement	486
		Percent Agreement	100%

Robustness

Planned deviation analysis:

		Average OD Values		
	O.N. coating / 2 hour sample	4 hour coating / 2 hour sample	4 hour coating / O.N. sample	O.N. coating / O.N. sample
Positive Sample #1 (High)	3.698	3.671	3.857	3.857
Positive Sample #1 (Low)	0.258	0.265	0.549	0.510
Positive Sample #2 (High)	3.455	3.279	3.827	3.842
Positive Sample #2 (Low)	0.357	0.370	0.769	0.747
Positive Sample #3 (High)	1.207	1.150	2.312	2.537
Negative Sample #1	0.090	0.089	0.092	0.100
Negative Sample #2	0.099	0.092	0.101	0.099
Negative Sample #3	0.108	0.095	0.092	0.101
Negative Sample #4	0.088	0.096	0.093	0.088
Negative Sample #5	0.094	0.091	0.088	0.092
Buffer	0.098	0.093	0.094	0.097



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Stability:

	1-year stability (accelerated)	1-year stability (real time)
Positive Sample (High)	Pass	Monitoring
Positive Sample (Medium)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Negative Sample #1	Pass	Monitoring
Negative Sample #2	Pass	Monitoring
Buffer	Pass	Monitoring
Negative Control	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:

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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