

Tech Note: ToBRFV ELISA Protocol and Performance Summary

PSA/SRA 66800 • Tomato brown rugose fruit virus (ToBRFV) ELISA

Protocol Notes

Seed Testing & Extraction Buffers: This ELISA was developed for the specific detection of Tomato brown rugose fruit virus (ToBRFV). This assay is fully compatible with the ISHI-Veg Test Protocols for the Detection of Infectious Tobamoviruses for Tomato Seed ([Version 6.2](#)) and Pepper Seed ([Version 6.3](#)) as an ELISA pre-screen. Internal testing indicates optimal test sensitivity is achieved when using PBS buffer detailed in the ISHI-Veg Test Protocols as the extraction buffer for tomato and pepper seed.

Enzyme Conjugate Diluent: This test is fully compatible with Agdia's ECI conjugate diluent and the conjugate diluent detailed in the ISHI-Veg Test Protocols. Furthermore, a 10-15% increase in sensitivity may be achieved by using Agdia's optional RUB6 enzyme conjugate diluent.

Specificity Testing: Tobamovirus cross-reactivity profile

The following foliar samples naturally infected with a high titer of various Tobamovirus species were tested against the new ToBRFV ELISA. No cross-reactivity was observed outside of slight optical density (OD) value elevation with one Tobacco mosaic virus (TMV) sample.

Note: OD values in Table 1 represent averages of the three replicates run per sample.

*Sample dilution range for ToMV-2 was 1:10 to 1:7290

Sample Dilution	TMGMV	PMMoV	ToMV-1	TMV-1	CGMMV	TMV-2	ToMV-2*
1: 1	0.100	0.108	0.092	0.140	0.098	0.127	0.095
1: 3	0.093	0.089	0.097	0.144	0.089	0.116	0.099
1: 9	0.096	0.092	0.094	0.146	0.097	0.106	0.104
1: 27	0.095	0.091	0.095	0.133	0.092	0.104	0.095
1: 81	0.098	0.094	0.106	0.131	0.106	0.106	0.099
1: 243	0.101	0.089	0.095	0.114	0.091	0.097	0.097
1: 729	0.096	0.096	0.091	0.102	0.095	0.094	0.090
Buffer	0.098	0.095	0.092	0.094	0.101	0.090	0.094

Sensitivity:

Agdia's ELISA for detection of Tomato brown rugose fruit virus (ToBRFV, Tobamovirus) demonstrates improved analytical sensitivity compared to Agdia's TMV ELISA. Notably, Agdia's TMV ELISA has been widely used as the preferred serological method to detect ToBRFV since the beginning of the current outbreak. Table 2 illustrates the sensitivity differences between the two assays. Optical density values shown in Table 2 represent averages of the three replicates run per sample. Isolates of ToBRFV from around the world were tested with the new ELISA and all were detected. The most up to date information about isolates tested can be found on the product webpage.

Sample Dilution	TMV ELISA Cat# 57400	ToBRFV ELISA Cat# 66800
1: 30	2.587	3.892
1: 90	1.881	3.775
1: 270	1.119	2.442
1: 810	0.645	1.256
1: 2430	0.341	0.540
1: 7290	0.211	0.252
1: 21870	0.158	0.143
Buffer	0.120	0.098

For answers to your technical questions, please contact us at techsupport@agdia.com.



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