

**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION  
 ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES  
 Summary sheet of validation data for a diagnostic test**

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

<b>Laboratory contact details</b>	National Reference Centre, National Plant Protection Organization P.O. Box 9102, 6700 HC Wageningen, Netherlands
<b>Short description of the test</b>	Detection of Tomato brown rugose fruit virus by DAS-ELISA in leaves and seeds of tomato
<b>Date, reference of the validation report</b>	2021-12-01 - Euphresco 2019-A-327 project report
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	yes
<b>Was the validated data generated in the framework of a project?</b>	Euphresco
<b>If yes, please specify</b>	Euphresco 2019-A-327
<b>Description of the test</b>	
<b>Organism(s)</b>	Tomato brown rugose fruit virus(TOBRFV)
<b>Detection / identification</b>	detection
<b>Matrix(ces) tested</b>	Leaves, Seeds
<b>Plant species tested</b>	Solanum lycopersicum
<b>Method(s)</b>	Extraction Serological DAS-ELISA Serological DAS-ELISA (2) Serological DAS-ELISA (3) Serological DAS-ELISA (4)
<b>Method: Extraction</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPPO diagnostic protocol?</b>	
<b>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>Name of the test</b>	
<b>As or adapted from an IPPC diagnostic protocol</b>	no

Is the test modified compared to the reference test	no
<b>Other information</b>	
Other details on the test	Leaves or seeds were ground in extraction buffer using extraction bags (Bioreba) and a hand-held homogeniser (Bioreba).
<b>Method: Serological DAS-ELISA</b>	
<b>Reference of the test description</b>	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	
Is the test modified compared to the reference test	
<b>Kit</b>	
Is a kit used	yes
Manufacturer name	PRIME DIAGNOSTICS
Specify the kit used	DAS-ELISA diagnostic kit for the detection of ToBRFV
Kit used following the manufacturer's instructions?	See report
<b>Other information</b>	
Reaction type	
Other details on the test	
<b>Method: Serological DAS-ELISA (2)</b>	
<b>Reference of the test description</b>	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	
Is the test modified compared to the reference test	
<b>Kit</b>	
Is a kit used	yes
Manufacturer name	LOEWE
Specify the kit used	Tomato Brown Rugose Fruit Tobamovirus. TBRFV DAS ELISA Cat No 07175

Kit used following the manufacturer's instructions?	see report
<b>Other information</b>	
Reaction type	
Other details on the test	
<b>Method: Serological DAS-ELISA (3)</b>	
<b>Reference of the test description</b>	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	
Is the test modified compared to the reference test	
<b>Kit</b>	
Is a kit used	yes
Manufacturer name	AGDIA
Specify the kit used	ELISA Reagent Set for ToBRFV (SRA 66800)
Kit used following the manufacturer's instructions?	see report
<b>Other information</b>	
Reaction type	
Other details on the test	
<b>Method: Serological DAS-ELISA (4)</b>	
<b>Reference of the test description</b>	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	
Is the test modified compared to the reference test	
<b>Kit</b>	
Is a kit used	yes
Manufacturer name	DSMZ
Specify the kit used	ToBRFV - DAS-ELISA kit (RT-1236)
Kit used following the manufacturer's instructions?	see report
<b>Other information</b>	

<b>Reaction type</b>	
<b>Other details on the test</b>	
<b>Are the performance characteristics included in the EPPO diagnostic protocol?</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Tomato brown rugose fruit virus(TOBRFV)</b>
<b>Analytical sensitivity</b>	
<b>What is smallest amount of target that can be detected reliably?</b>	With samples consisting of extract from uninfected tomato leaves, and serial dilutions of virus infected leaf material in uninfected tomato leaf extract: ToBRFV was detected up to a dilution of 10 <sup>-4</sup> (Loewe, DSMZ, Prime Diagnostics) or 10 <sup>-5</sup> (Agdia). With samples of infected seeds: The highly infected samples were barely detected by modified DSMZ test and clearly detected by modified Prime Diagnostics test. The medium infected samples were not or only barely detected by both modified tests.
<b>Diagnostic sensitivity</b>	
<b>Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98</b>	
<b>Standard test(s)</b>	
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	
<b>Specificity value</b>	
<b>Analytical specificity - exclusivity</b>	
<b>Number of non-target organisms tested</b>	Samples consisting of extract from uninfected tomato leaves, and serial dilutions of virus infected leaf material in uninfected tomato leaf extract: TMV (x2 isolate) ToMV (x1 isolate)
<b>Specificity value</b>	Cross-reactions were observed for Loewe with ToMV (up to 10 <sup>-1</sup> ), for Agdia with all tested isolates (generally up to 10 <sup>-5</sup> ), for DSMZ with all tested isolates (depending on the isolate ranging from undiluted up to 10 <sup>-3</sup> ) and for Prime Diagnostics with TMV isolate 1 (undiluted) and ToMV (up to 10 <sup>-3</sup> ).
<b>Cross reacts with</b>	
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	
<b>Specify the test(s)</b>	
<b>Reproducibility</b>	

<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Brief details of the test performance study and its output. It available, link to published article/report</b>	
<b>Other information</b>	
<b>Any other information considered useful</b>	The report also contains a comparison between extraction buffer from manufacturer and extraction buffer from NVWA
The following complementary files are available online:	<ul style="list-style-type: none"> <li>• <a href="#">Report_2019-A-327_Euphresco</a></li> </ul>

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