

**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>	Netherlands Institute for Vectors, Invasive plants and Plant health 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 / Tobamovirus fructirugosum (TOBRFV)
<b>Detection / identification</b>	detection
<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>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Is the test modified compared to the reference test</b>	no
<b>Other information</b>	
<b>Other details on the test</b>	Leaves or seeds were ground in extraction buffer using extraction bags (Bioreba) and a hand-held homogeniser (Bioreba).

<b>Method: Serological DAS-ELISA</b>	
<i>Reference of the test description</i>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	PRIME DIAGNOSTICS
<b>Specify the kit used</b>	DAS-ELISA diagnostic kit for the detection of ToBRFV
Kit used following the manufacturer's instructions?	See report
<i>Other information</i>	
<b>Method: Serological DAS-ELISA (2)</b>	
<i>Reference of the test description</i>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	LOEWE
<b>Specify the kit used</b>	Tomato Brown Rugose Fruit Tobamovirus. TBRFV DAS ELISA Cat No 07175
Kit used following the manufacturer's instructions?	see report
<i>Other information</i>	
<b>Method: Serological DAS-ELISA (3)</b>	
<i>Reference of the test description</i>	
<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	AGDIA
<b>Specify the kit used</b>	ELISA Reagent Set for ToBRFV (SRA 66800)
Kit used following the manufacturer's instructions?	see report
<i>Other information</i>	
<b>Method: Serological DAS-ELISA (4)</b>	
<i>Reference of the test description</i>	

<b>As or adapted from an EPPO diagnostic protocol</b>	no
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	DSMZ
<b>Specify the kit used</b>	Tomato brown rugose fruit virus (DAS-ELISA) ToBRFV (RT-1236)
Kit used following the manufacturer's instructions?	see report
<b>Other information</b>	
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Tobamovirus fructirugosum(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>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>Test performance study</b>	
<b>Test performance study?</b>	no
<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>

