

**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>	Validation of the Menzel & Winter real-time RT-PCR for the detection and identification of tomato brown rugose fruit virus
<b>Date, reference of the validation report</b>	2023-02-06 - 2021.molbio.012
<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>	no
<b>Description of the test</b>	
<b>Organism(s)</b>	Tomato brown rugose fruit virus / Tobamovirus fructirugosum (TOBRFV)
<b>Detection / identification</b>	detection and identification
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time RT PCR
<b>Method: Molecular Extraction DNA RNA</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>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	QIAGEN
<b>Specify the kit used</b>	RNeasy Plant Mini Kit
<b>Kit used following the manufacturer's instructions?</b>	no see validation report

<b>Other information</b>	
<b>Method: Molecular real time RT PCR</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>	no
<b>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 1)
<b>Name of the test</b>	Real-time RT-PCR Menzel and Winter (2021)
<b>Is the test modified compared to the reference test</b>	no
<b>Kit</b>	
<b>Is a kit used</b>	yes
<b>Manufacturer name</b>	Applied Biosystems
<b>Specify the kit used</b>	TaqMan RNA-to-CT 1-Step Kit
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Simplex - Probe
<b>Performance Criteria :</b>	
<b>Organism 1.:</b>	<b>Tobamovirus fructirugosum(TOBRFV)</b>
<b><u>Analytical sensitivity</u></b>	
<b>What is smallest amount of target that can be detected reliably?</b>	Relative dilution of 10 <sup>10</sup> .
<b><u>Analytical specificity - inclusivity</u></b>	
<b>Number of strains/populations of target organisms tested</b>	33 ToBRFV isolates tested 137 ToBRFV isolates in silico
<b>Specificity value</b>	100%
<b><u>Analytical specificity - exclusivity</u></b>	
<b>Number of non-target organisms tested</b>	Several Solanum lycopersicum and Capsicum sp. infecting tobamoviruses. See report.
<b>Specificity value</b>	
<b><u>Reproducibility</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100
<b><u>Repeatability</u></b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	100
<b>Test performance study</b>	
<b>Test performance study?</b>	no

The following complementary files are available online:	<ul style="list-style-type: none"><li>• <a href="#">2021.molbio.012 Validation real-time PCR Menzel &amp; Winter ToBRFV V2.0</a></li></ul>

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