

**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.

Laboratory contact details	Netherlands Institute for Vectors, Invasive plants and Plant health P.O. Box 9102, 6700 HC Wageningen, Netherlands
Short description of the test	Validation of the Menzel & Winter real-time RT-PCR for the detection and identification of tomato brown rugose fruit virus
Date, reference of the validation report	2023-02-06 - 2021.molbio.012
Validation process according to EPPO Standard PM7/98?	yes
Is the lab accredited for this test?	yes
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Tobamovirus fructirugosum(TOBRFV)
Detection / identification	detection and identification
Method(s)	Molecular Extraction DNA RNA Molecular real time RT PCR
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	no
EPPO Diagnostic Protocol name	PM 7/146 Tomato brown rugose fruit virus (version 1)
Is the test modified compared to the reference test	no
Kit	
Is a kit used	yes
Manufacturer name	QIAGEN
Specify the kit used	RNeasy Plant Mini Kit
Kit used following the manufacturer's instructions?	no see validation report
Other information	

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

The following complementary files are available online:

- [2021.molbio.012 Validation real-time PCR Menzel & Winter ToBRFV V2.0](#)

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