

**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 Institute of Biology, Department of Biotechnology and Systems Biology Vecna pot 121, 1000 Ljubljana, Slovenia
<b>Short description of the test</b>	Validation report on the testing of tomato brown rugose fruit virus by ABIOPEP real-time RT-PCR.
<b>Date, reference of the validation report</b>	2023-12-14 - Validation report on the testing of tomato brown rugose fruit virus by ABIOPEP real-time RT-PCR
<b>Validation process according to EPPO Standard PM7/98?</b>	yes
<b>Is the lab accredited for this test?</b>	no
<b>Was the validated data generated in the framework of a project?</b>	EURL
<b>If yes, please specify</b>	EURL-Virology (European Union Reference Laboratory for pests of plants on viruses, viroids and phytoplasmas)
<b>Description of the test</b>	
<b>Organism(s)</b>	Tomato brown rugose fruit virus(TOBRFV)
<b>Detection / identification</b>	detection and identification
<b>Method(s)</b>	Molecular Extraction DNA RNA Molecular real time RT PCR Molecular real time RT PCR (2) Molecular real time RT PCR (3)
<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>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 2)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<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
Kit used following the manufacturer's instructions?	yes For seed extraction the kit was used with the following modifications: the RLT buffer was replaced by GH+ buffer (EPPO PM7/146(2) Appendix 1) and the centrifugation temperature was decreased to 4°C at all steps to optimize RNA extraction from seed.
<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>EPPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 2)
<b>Name of the test</b>	Real-time RT-PCR Bernabé-Orts et al. (2021)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<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>	ThermoFisher Scientific
<b>Specify the kit used</b>	TaqManR RNA-to-Ct™ 1-Step Kit
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Method: Molecular real time RT PCR (2)</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 2)
<b>Name of the test</b>	Real-time RT-PCR Bernabé-Orts et al. (2021)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<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>	ThermoFisher Scientific
<b>Specify the kit used</b>	AgPath-ID™ One-Step RT-PCR
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<b>Method: Molecular real time RT PCR (3)</b>	
<b>Reference of the test description</b>	
<b>As or adapted from an EPO diagnostic protocol</b>	yes
<b>New test being considered for inclusion in the next version of the EPO diagnostic protocol?</b>	no
<b>EPO Diagnostic Protocol name</b>	PM 7/146 Tomato brown rugose fruit virus (version 2)
<b>Name of the test</b>	Real-time RT-PCR Bernabé-Orts et al. (2021)
<b>As or adapted from an IPPC diagnostic protocol</b>	no
<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>	KAPA BIOSYSTEMS
<b>Specify the kit used</b>	KAPA PROBE FAST Universal One-Step qRT-PCR Kit
Kit used following the manufacturer's instructions?	yes
<b>Other information</b>	
<b>Reaction type</b>	Simplex
<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>	Dilutions of ToBRFV infected tomato leaves in sap from healthy leaves. LOD 10 <sup>-6</sup> .
<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>	Number of targets tested: 29 (seed samples) Number of non-targets tested: 11 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 2 Tomato seeds: 95.7% Pepper seeds: 88.9%
<b>Analytical specificity - inclusivity</b>	
<b>Number of strains/populations of target organisms tested</b>	Number of targets tested: 7 (ToBRFV isolates)
<b>Specificity value</b>	100%
<b>Analytical specificity - exclusivity</b>	

<b>Number of non-target organisms tested</b>	Number of non-targets tested: 19 (isolates of other tobamoviruses).
<b>Specificity value</b>	100%
<b>Diagnostic Specificity</b>	
<b>Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test</b>	Number of targets tested: 29 (seed samples) Number of non-targets tested: 11 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 2 Tomato seeds: 100% Pepper seeds: 100%
<b>Reproducibility</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Percentage of identical results is 100%. No. of target samples tested: 2 (RNA samples). No. of different days: 5
<b>Repeatability</b>	
<b>Provide the calculated % of agreement for a given level of the pest (see PM 7/98)</b>	Not evaluated.
<b>Test performance study</b>	
<b>Test performance study?</b>	no
<b>Other information</b>	
<b>Any other information considered useful</b>	Additionally Robustness of the test was evaluated: Percentage of correct results is 100% Number of targets tested: 2 (seed samples) Number of non-targets tested: 5 (seed samples) Number of laboratories included in the evaluation of these performance characteristics: 9 Number of different RNA extraction: 3 Number of different reagents for real-time RT-PCR: 5 Number of different instruments: 5 Full validation report is available on the EURL webpage: <a href="https://eurlplanthealth.nl/files/view/e7dde713-181c-4363-aabe-031aa39873e7/20231214_tomato_brown_rugose_fruit_virus_validation_real-time_rt-pcr_bernabe-orts-et-al.pdf">https://eurlplanthealth.nl/files/view/e7dde713-181c-4363-aabe-031aa39873e7/20231214_tomato_brown_rugose_fruit_virus_validation_real-time_rt-pcr_bernabe-orts-et-al.pdf</a>

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