

# Validation report

## qPCR ToBRFV sets

Article No.: 889600 (qPCR ToBRFV set 96) / 889200 (qPCR ToBRFV set 192)

### General information:

Target Pathogen	ToBRFV (tomato brown rugose fruit virus, Jordan-virus)
Genus	<i>Tobamovirus</i>
Method	Real-Time RT-qPCR, TaqMan

### Technical information:

Fluorophores	FAM: ToBRFV   HEX: ToBRFV   ROX: COX
Cycling program	<ul style="list-style-type: none"> <li>➤ 50°C for 20 min (Reverse Transcription)</li> <li>➤ 95°C for 5 min (RT inactivation)</li> <li>➤ 40 Cycles: <ul style="list-style-type: none"> <li>○ 95°C for 15 sec (Denaturation)</li> <li>○ 60°C for 30 sec (Annealing / Extension)</li> </ul> </li> </ul>
Controls	<p>Internal positive control (IPC): cytochrome oxidase (COX)</p> <p>Negative control (NC): plant DNA from leaf, seed, or fruit</p> <p>Positive control (PC): ToBRFV-infected plant extracts (<u>not included</u>)</p>

### Host matrix:

Tested plant material	Leaf, fruit, seed Seed transmittable / mechanical transmittable
Tested species infected	<i>Solanum lycopersicum</i> (Tomato) <i>Capsicum</i> spp. (Peppers)

### Specificity:

Analytical Specificity	100%
Number of tested samples from target organism (True Positives)	54
Diagnostic Specificity	100%   PT: 100%
Number of tested samples non-target organism (True Negatives)	>100
Accuracy	100%   PT: 100%
Detected isolates / geographic regions (Inclusivity)	PV-1236 (Germany, Tomato) PV-1244 (Germany, Tomato) PV-1241 (Israel, Tomato) PV-1278 (Netherlands, Tomato) IT-01 (Italy, Tomato) INF.110721-A (Israel, Tomato) INF.110721-B (Israel, Tomato) INF.110721-C (Israel, Tomato) INF.110721-D (Israel, Tomato) INF.110721-E (Israel, Tomato) INF.110721-F (Israel, Tomato) INF.130721-A (Israel, Pepper) INF.130721-B (Israel, Pepper) INF.130721-C (Israel, Pepper) INF.130721-D (Israel, Pepper)



	Tomato-09-21 (Switzerland, 2021)
Cross reaction with (Exclusivity)	None known
No cross reaction tested with (Exclusivity)	ToMV (Tomato mosaic virus) TMV (Tobacco mosaic virus) PMMoV (Pepper mild mottle virus) CGMMV (Cucumber green mottle mosaic virus) TSWV (Tomato spotted wilt virus) TBRV (Tomato black ring virus) TBSV (Tomato bushy stunt virus) TYLCV (Tomato yellow leaf curl virus) PepMV (Pepino mosaic virus) ToRSV (Tomato ringspot virus) CMV (Cucumber mosaic virus) INSV (Impatiens necrotic spot virus) TNV (Tobacco necrosis virus) ToANV (Tomato apex necrosis virus) TSV (Tobacco streak virus)
No matrix effect observed with (Selectivity)	<i>Solanum lycopersicum</i> (Tomato) <i>Capsicum annuum</i> (Chili/Paprika) <i>Capsicum</i> spp. (Peppers) <i>Nicotiana benthamiana</i> (Tobacco) <i>Nicotiana clevelandii</i> (Tobacco) <i>Chenopodium Quinoa</i> (Quinoa) <i>Solanum tuberosum</i> (Potato) – Tuber and Leaf

**Sensitivity:**

Diagnostic Sensitivity	100%   PT: 100%
Analytical Sensitivity / LoD	10 <sup>-6</sup> to 10 <sup>-8</sup>
Sensitivity on host matrix	Tomato leaves: 10 <sup>-6</sup> to 10 <sup>-8</sup> Tomato seeds: 10 <sup>-6</sup> to 10 <sup>-8</sup> Tomato fruit: 10 <sup>-6</sup> to 10 <sup>-7</sup> Pepper leaves: 10 <sup>-6</sup> to 10 <sup>-7</sup>
Other sensitivity characteristics	One seed lot of ToBRFV infected tomato seeds was mixed with healthy tomato seeds: <ul style="list-style-type: none"> <li>Seed lot standard: <b>Ct 15.9</b></li> <li>Seed lot 1:250: <b>Ct 19.4</b></li> <li>Seed lot 1:1000: <b>Ct 23.7</b></li> <li>Seed lot 1:3000: <b>Ct 27.6</b></li> </ul> <p>500 healthy pepper seeds were contaminated with 3 ToBRFV-infected seeds: <b>Ct 22.3</b></p> <p>1000 healthy tomato seeds were contaminated with 3 ToBRFV-infected seeds: <b>Ct 18.1</b></p> <p>1000 healthy tomato seeds were contaminated with 5 ToBRFV-infected seeds: <b>Ct 18.5</b></p>

**Validation:**

Internal validation	01.10.2020 – 31.10.2021
External validation	01.07.2021 – 31.10.2021 1 proficiency test (PT) in 2023 (Italy)
Reproducibility	100%

<b>Repeatability</b>	100%
<b>Validation information</b>	<p>One validation was done in a BIOREBA performance study (PS) with a laboratory in Israel. ToBRFV-infected seed samples provided by the external laboratory were tested with the same PCR-Method in both laboratories.</p> <p>One validation was done with external, commercially available isolates (reference material).</p> <p>In 2023 BIOREBA participated on a proficiency test (PT) in Italy.</p>

Validation release Date:  
November, 3<sup>rd</sup>, 2021

QC manager:




Version: 3 – 19.07.2023 – Adaptations from last version: Update with new data and information and additional information from PT added.