

# Validation report

## DAS-ELISA ToBRFV

Article No.: 152877 (ToBRFV Complete kit 96) / 152875 (ToBRFV Complete kit 480) / 152872 (ToBRFV Complete kit 960)

### General information:

Target Pathogen	ToBRFV (Tomato brown rugose fruit virus)
Genus	<i>Tobamovirus</i>
Method	DAS-ELISA

### Technical information:

Antibodies	Polyclonal antibodies developed against a ToBRFV isolate from tomato (origin: Germany)
Sampling	Leaf samples: 1:20 (w/v) in extraction buffer "general" Fruit samples: 1:20 (w/v) in extraction buffer "general" Seed samples: 1:25 (w/v) in extraction buffer "general"
Controls	Negative control (NC): lyophilized extracts from healthy plants Positive control (PC): "Tobamovirus Positive control", lyophilized plant extracts infected with a <i>tobamovirus</i>
Working volume	200 µl / well

### 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	99.07%
Number of tested samples from target organism (True Positives)	31
Diagnostic Specificity	100%
Number of tested samples non-target organism (True Negatives)	130
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)	TMV (Tobacco mosaic virus) ToMV (Tomato mosaic virus) PMMoV (Pepper mild mottle virus) / weakly
No cross reaction tested with (Exclusivity)	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 annum</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 muricatum</i> (Pepino)

**Sensitivity:**

Analytical Sensitivity / LoD	100% ( $10^{-4}$ to $10^{-7}$ )
Sensitivity on host matrix	Tomato leaves: $10^{-6}$ Pepper leaves: $10^{-6}$ Tomato fruit: $10^{-4}$ Tomato seeds: $10^{-5}$
Other sensitivity characteristics	PV-1236: $1.4 \cdot 10^{-6}$ PV-1244: $4.9 \cdot 10^{-5}$ PV-1241: $1.6 \cdot 10^{-5}$ PV-1278: $1.6 \cdot 10^{-5}$

**Validation:**

Internal validation	01.11.2020 – 31.05.2021 01.08.2021 – 30.09.2021
External validation	01.07.2021 – 30.07.2021
Reproducibility	100% (BIOREBA +1)
Repeatability	100% (BIOREBA +1)
Validation information	One validation was done in a BIOREBA performance study (PT) with a laboratory in Israel. The BIOREBA DAS-ELISA ToBRFV complete kit was used in both laboratories with samples provided by the external laboratory.  One validation was done with external, commercially available isolates (reference material).

Validation release Date:  
November, 2<sup>nd</sup>, 2021

QC manager:



Version: 2 – 13.07.2022 – Adaptations from last version: redesign of the validation report structure.