

## PhytoDETECT™

### Clavibacter michiganensis subsp. michiganensis qPCR Kit

#### Bacterial Canker of Tomato Detection by qPCR

TBK1121-50. 50 reactions

TBK1121-100. 100 reactions

TBK1121-500. 500 reactions

#### Introduction

PhytoDETECT™ *Clavibacter michiganensis* subsp. *michiganensis* qPCR Kit enables accurate detection of this bacteria through a real-time quantitative PCR reaction. The kit includes a master mix containing the necessary enzymes, optimized primers and probes, as well as a DNA-based positive amplification control (PAC) to ensure that the PCR amplification is performed efficiently with the supplied components.

*Clavibacter michiganensis* subsp. *michiganensis* (Cmm) is a Gram-positive, non-sporulating, slow-growing bacterium belonging to the actinobacteria group. It is the causal agent of black canker of tomato, a disease characterized by unilateral wilting, stem cankers, vascular necrosis, and “bird’s-eye” lesions on fruit. Cmm is highly pathogenic and regarded as a quarantine organism in several regions due to its efficient transmission through infected seeds and contaminated plant debris.

#### Features

- Cmm detection in FAM channel.
- Compatible with all real-time thermocyclers.

#### Kit Components

Components	50 rxn	100 rxn	500 rxn
qPCR Probe Master Mix (2x)	0.5 mL	1 mL	5x 1 mL
ROX Reference	1 vial	1 vial	1 vial
Cmm Primers & Probe Mix (10x)	100 µL	200 µL	1 mL
Cmm_PAC (Positive Control)	1 vial	1 vial	1 vial
Water, nuclease free	1 mL	1 mL	3x 2 mL

**Order Info Kit Components:** qPCR Probe Master Mix (TBZ0350) | ROX Reference (TBRO278) | Cmm Primers-& Probe Mix (10x) (TBK1121-1) | Cmm\_PAC (TBK1121-2) | Water, nuclease free (TBB0302).

#### Storage

PhytoDETECT™ *Clavibacter michiganensis* subsp. *michiganensis* qPCR Kit is shipped with cold gel packs. Upon receipt, store the kit at -20°C. Avoid repeated freeze-thaw cycles. The Cmm Primers & Probe Mix is light-sensitive and should be stored in the dark.

#### Required Materials (not included)

- Filter tips
- Optical-grade PCR tubes/plates
- Optical sealing film

**Technical Support:** [info@tiarisbiosciences.com](mailto:info@tiarisbiosciences.com)

## PROTOCOL

### A. Real Time Quantitative PCR

1. Thaw all kit components on ice. Mix each solution thoroughly and briefly spin down the tubes.
2. Use the following reaction setup for a 20  $\mu$ L reaction volume:

Components	Reaction Volume*
qPCR Probe Master Mix (2x)	10 $\mu$ L
Cmm Primers & Probe Mix (10x)	2 $\mu$ L
Water, nuclease free	Up to 15 - 18 $\mu$ L

\* Prepare a mix for all reactions, considering two additional reactions for controls. Use ROX if it is required by the thermocycler.


3. Distribute **15-18  $\mu$ L of the prepared mix** into the required number of tubes/wells. Include one well for NAC and one for PAC (see notes).

Use 5  $\mu$ L of Cmm\_PAC dilution (1:10) (Positive Amplification Control).

4. Add **2-5  $\mu$ L of extracted DNA sample** to each reaction tube and mix well.

The quality of the test depends on the quality of the DNA sample. Improper collection, storage, or transport of samples can lead to false negatives.

5. Place the tubes in the thermocycler and set up the following real-time PCR program:

Step	Temperature	Time	Cycles	Detection
Initial Activation	95 °C	3 min	1x	
Denaturation*	95 °C	10 sec	35-40x	
Annealing & Extension	60 °C	30 sec		

\* Ramp 5°C/second

### B. Amplification Monitoring & Data Analysis

1. To monitor amplification in real-time, fluorescence should be measured in the **FAM channel** (Excitation 495 nm / Emission 520 nm), following the thermocycler's user manual. Results should be interpreted as follows:

	Cmm Presence	Cmm Absence
<b>PAC (Positive Control)</b>	+	+
<b>NAC (Negative Control)</b>	C <sub>T</sub> = N/A	C <sub>T</sub> = N/A
<b>Sample</b>	C <sub>T</sub> < 35	C <sub>T</sub> $\geq$ 35

**Notes**

- Positive Amplification Control (PAC): Ensures PCR efficiency. **PhytoDETECT™ Cmm qPCR Kit** includes a DNA-based Cmm\_PAC.
- Negative Amplification Control (NAC): Prevents false positives due to contamination. Use nuclease-free molecular biology water.