# PhytoDETECT™ PepMV RT-qPCR Kit

(Pepino Mosaic Virus (PepMV) Detection by RT-qPCR)

TBK1080-50. 50 reactions

TBK1080-100. 100 reactions

## Introduction

PhytoDETECT™ PepMV RT-qPCR Kit enables the detection of pepino mosaic virus (PepMV) through a real-time quantitative RT-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.

The pepino mosaic virus is a pathogen belonging to the *Potexvirus* family that represent a serious threat to tomato crops globally and is regulated as a quarantine pest in Europe. Characterized by causing a variety of symptoms such as mosaic, leaf distortion, and stunted growth, PepMV can spread rapidly in greenhouse and open field conditions through mechanical contact and crop management practices. This diagnostic kit provides a reliable and efficient solution for the detection of PepMV, facilitating the timely adoption of preventive measures in agricultural production.

## Features

- One-tube cDNA synthesis and PCR reaction
- Compatible with all real-time thermocyclers
- PepMV detection in FAM channel

## Kit Components

| Components | 50 rxn | 100 rxn |
|---|---|---|
| qPCR Probe Master Mix (2x) | 0.5 mL | 1 mL |
| ROX Reference | 1 vial | 1 vial |
| RT Mix | 50 µL | 100 µL |
| PepMV Primers & Probe Mix (10x) | 100 µL | 200 µL |
| PepMV_PAC (Positive Control) | 1 vial | 1 vial |
| Water, nuclease free | 1 mL | 1 mL |

***Order Info Kit Components:*** *qPCR Probe Master Mix (TBZ0350) | ROX Reference (TBR0278) | RT-Mix (TBZ0352) |* PepMV Primers & Probe Mix (10x) *(TBK1080-1) | PepMV_PAC (TBK1080-2) | Water, nuclease free (TBB0302).*

## Storage

PhytoDETECT™ PepMV RT-qPCR Kit is shipped with cold gel packs. Upon receipt, store the kit at -20°C. Avoid repeated freeze-thaw cycles. The PepMV 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

www.tiarisbiosciences.com

## PROTOCOL

### Technical Recommendations

- *RNA extraction is **mandatory** before using the **PhytoDetect™ PepMV RT-qPCR Kit**.*
- *The quality of the extracted RNA significantly impacts the overall assay performance. Ensure that the nucleic acid extraction system used is compatible with RT-qPCR.*
- *Include an **internal extraction control** when performing RNA extraction.*

## A. RT-qPCR

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 µL reaction volume:

| Component | Reaction Volume* |
|---|---|
| qPCR Probe Master Mix (2x) | 10 µL |
| RT Mix | 1 µL |
| PepMV Primers & Probe Mix (10x) | 2 µL |
| Water, nuclease free | Up to 15 - 18 µL |

* *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 µL of the prepared mix** into the required number of tubes/wells. Include one well for NAC and one for PAC (*see notes*).
   *Use **5 µL of a PepMV_PAC dilution (1:10)** (Positive Amplification Control).*
4. Add **2-5 µL of extracted RNA** sample to each reaction tube and mix well.
   *The quality of the test depends on the quality of the RNA 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 |
|---|---|---|---|---|
| Reverse Transcription | 50 °C | 20 min | 1x | |
| Initial Activation | 95 °C | 5 min | 1x | |
| Denaturation | 95 °C | 5 sec | 35x | |
| Annealing & Extension | 59 °C | 25 sec | | 📷 |

## 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:

| | **PepMV Presence** | **PepMV Absence** |
|---|---|---|
| **PAC** (Positive Control) | + | + |
| **NAC** (Negative Control) | $C_T$= N/A | $C_T$= N/A |
| **Sample** | $C_T \leq 33$ | $C_T > 33$ |

*Notes*

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