User Guide
# PCR Pepino mosaic virus kit

*Version 03 – 20/09/2021*

## Information pest: Pepino mosaic virus

Pepino mosaic virus (PepMV) is a regulated plant pathogen included in European legislation as a quarantine pest.

PepMV was first described from pepino in 1980 in Peru. Since 1999, PepMV started infecting tomato crops with rapid and worldwide spread. Four major strain groups are distinguished: European (EU), Peru, Ch2 and US1. PepMV can be detected on growing plants (tomato, pepino), on tomato fruits and on tomato seeds. Symptoms can be extremely variable, ranging from latent to very severe infections (fruit discolorations, fruit cracking and malformation).

## Introduction

The PCR Pepino mosaic virus kit has been developed by Qualiplante based on Ling et al. (2008). The primer pair was designed on the TGB2-3 genomic region.

The PCR PepMV kit enables the detection of the 4 strain groups: European (EU), US1, US2 and Ch2.

Validation data of the method are available from a study realized in 2012 during Progetto Aron-Arnadia - Armonizzazione Protocollo diagnostico per Pepino mosaic virus (PepMV) su pomodoro, Progetto Strateco. The performance characteristics obtained are:

- Diagnostic sensitivity: 91%.
- Diagnostic specificity: 100%.
- Accuracy: 93%.
- Analytical sensitivity: $10^{-5}$ for seeds, $10^{-8}$ for fruit pulp, $10^{-6}$ for leaves.
- Analytical specificity: 100%.
- Repeatability: 100%.
- Reproducibility: 98%.

*This product should be used only for research purposes.*

## Intended use

The PCR kit is validated for the detection of Pepino mosaic virus (PepMV) in One-Step End-Point RT-PCR.

Suitable tissues are seeds, leaves and fruit pulp of tomato plants.

## Kit format and content

| Article N° | Product name |
|---|---|
| PCR PepMV 96 | PCR Pepino mosaic virus<br>96 tests |

| Content | 96 tests |
|---|---|
| Direct Master Mix | 2x48 tests |
| RT-Enzyme | 96 tests |
| Positive Control | 8 tests |
| Negative Control | 8 tests |

## Storage conditions

This kit can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

## Shelf life

If the kit is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

## Materials and equipment (not provided)

- RNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- DNA ladder and loading-dye buffer
- PCR thermal cycling
- Agarose gel reagents and apparatus

## Nucleic acids extraction

Extract RNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

## Preparation of the PepMV 1-Step master mix

a) Slowly thaw Direct Master Mix and RT-Enzyme by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and RT-Enzyme and spin down the liquid.
c) In a new tube called **PepMV 1-Step master mix**, mix 18,5 µl of Direct Master Mix and 0,5 µl of RT-Enzyme per reaction. Do not forget to count the Positive Control and the Negative Control in the number of reactions to prepare.



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| Example: | 1 rxn | 10 rxns |
|---|---|---|
| Direct Master Mix | 18,5 µl | 185,0 µl |
| RT-enzyme | 0,5 µl | 5,0 µl |

d) Store the **PepMV 1-Step master mix** by placing it on ice or at 4°C.

## Reaction set-up

a) Shake briefly **PepMV 1-Step master mix** and spin down the liquid.
b) Add 19 µl of **PepMV 1-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
c) Add 1 µl of RNA template to the **PepMV 1-Step master mix**. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the Positive Control and the Negative Control.

| Components | Volume/PCR tube or well |
|---|---|
| RNA template or Positive control or Negative control | 1 µl |
| **PepMV 1-Step master mix** | 19 µl |
| Total Volume / PCR tube or well | 20 µl |

*In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.*

## Run and thermal cycling

a) Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
b) Load the PCR tubes or plate into the thermal-cycler and follow the thermal cycling below:

| Steps | Temp (°C) | Time | Cycle(s) |
|---|---|---|---|
| Reverse transcription | 50°C | 15 min | 1 |
| Enzyme activation | 95°C | 10 min | 1 |
| Denaturation | 95°C | 30 sec | 45 |
| Annealing and elongation | 60°C | 60 sec | |
| Storage | 4°C | ∞ | - |

## Agarose gel electrophoresis

Prepare an agarose gel **at 2% w/v in 1X-TBE buffer**.

*Gel loading*:
- load the DNA ladder (for example 100-1'000 bp DNA step ladder)
- load 10 µl of PCR products from the previous step adding the loading-dye buffer (not provided in the kit).

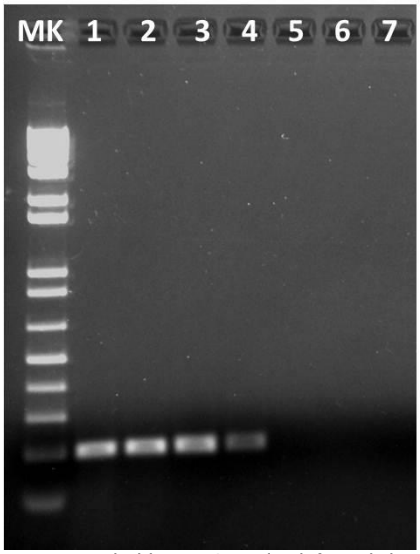
*Run*: run the gel electrophoresis for 50-60 minutes at 80V.

## Results analysis

### ANALYSIS VALIDATION

Pepino mosaic virus is detected when a 202 bp DNA fragment is observed. The analysis is validated when:
- ✓ 1 DNA fragment of 202 bp is visible in the positive control lane.
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 1X-TBE 2% agarose gel showing the RNA amplification in a sample infected by PepMV:




**MK:** DNA ladder - Sample infected by PepMV at different concentrations (or Positive control of the kit): **1:** 600 ng of total RNA - **2**: 100 ng of total RNA - **3**: 10 ng of total RNA - **4:** 1 ng of total RNA - **5:** 0,1 ng of total RNA - **6:** Healthy sample or Negative control of the kit - **7:** No template control.

### RESULTS INTERPRETATION

The specific product of PepMV is a 202 bp DNA fragment.

- ✓ A sample is **positive** when a 202 bp specific DNA fragment is present in the PCR reaction.
- ✓ A sample is **negative** when no fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

| Fragment size **202 bp** | Interpretation |
|---|---|
| - | Negative |
| ✓ | **POSITIVE** Pepino mosaic virus |



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## Special handling instructions

This kit was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA/RNA and the components of the kit. Do not touch any kit components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from kits with different batch numbers in the same test procedure. Do not interchange reagents with other kits. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the kit components. Do not use reagents after their expiration date.

## Troubleshooting

**Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:**

| Possible causes | Corrective actions |
|---|---|
| Evaporation of the sample due to inadequate sealing of the plate | Repeat the test using the appropriate tools to seal correctly the plate |
| Consumables are not appropriate for the method | Repeat the test using consumables recommended by the thermal cycler supplier |
| The quality of nucleic acid extracted is low | Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In case of doubt, contact us |
| Abnormal amplification | Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles |

**No amplification reaction is observed in the positive control well, while other samples are positive:**

| Possible causes | Corrective actions |
|---|---|
| The positive control provided with the kit was not added into the reaction well | Repeat the test. If the problem persists, contact us |

**An amplification plot is observed in the negative control well:**

| Possible causes | Corrective actions |
|---|---|
| Contamination of the negative control or the Master Mix with target-positive nucleic acid | Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly |

### Warranty and Responsibilities

*Qualiplante SAS guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits Qualiplante SAS responsibility to the replacement of the product. No other warranties, of any kind, express or implied-are provided by Qualiplante SAS.*
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