

User Guide qPCR Pepino mosaic virus kit

Version 07 – 03/12/2024

qualiplante

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 qPCR *Pepino mosaic virus* kit has been developed by Qualiplante based on Ling et al., 2007. A verification was performed by Qualiplante (data not published) and the performance characteristics of the kit are the same as the original publication.

The test was designed against the 5 strains of PepMV covered by 11 isolates: EU-tom, Ch-1, Ch-2, Peruvian and US-2 strains. The method was evaluated in a ring performance study on tomato seeds in 2009 as part of the EU-PRA PEPEIRA project. The test could detect one naturally infested seed in 5.000.

The One-Step Real-Time RT-PCR test of Ling et al., 2007 is referred in the Appendix 4 of the PM7/113 (1) Pepino mosaic virus European and Mediterranean Plant Protection Organization Bulletin (2013) 43 (1), 94-104 and recommended by Progetto Aron-Arnadia - Armonizzazione Protocollo diagnostico per Pepino mosaic virus (PepMV) su pomodoro, Progetto Strateco, Anno 2012.

This product should be used only for research purposes.

Intended use

The qPCR kit is validated for the detection of *Pepino mosaic virus* (PepMV) in One-Step Real-Time RT-PCR. Suitable tissues are leaves of tomato plants or tomato seeds. For reliability, subsamples of no more than 250 seeds should be used in an individual test.

Kit format and content

Article N°	Product name
qPCR PepMV 96	qPCR <i>Pepino mosaic virus</i> 96 tests
Content	96 tests
Nuclease free water	2 x 1,25 ml
Primers/probe mix	96 tests
Taq polymerase	2 x 48 tests
RT-Enzyme	2 x 48 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
- Thermal-cycler for Real-Time PCR with filters calibrated for FAM®

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-RT master mix

- Do a short spin in a centrifuge to collect the pellet at the bottom of the **Primers/probe mix** tube.
- Add 1.450,00 µl (one thousand four hundred fifty) of **nuclease free water** provided with the kit in the **Primers/probe mix** tube. Vortex vigorously up and down the **Primers/probe mix** tube. Spin down the liquid.
- Slowly thaw **Taq polymerase** and **RT-Enzyme** tubes by placing them on ice or at 4°C.
- Shake briefly **Taq polymerase** and **RT-Enzyme** tubes and spin down the liquid.
- For each reaction, in a new tube (not provided), prepare the **PepMV-RT Master mix** by mixing 4 µL of **Taq polymerase** and 0,5 µl of **RT-Enzyme** in 14,5 µl of **Primers/probe mix**. Do not forget the **Positive Control** and **Negative Control** of the kit which must be added to check that the reaction is working correctly.

Example: if you have 8 samples to test, you will need 10 reactions (8 samples + 1 **Positive Control** + 1 **Negative Control**) of 19 µl each. In a tube, please, mix 40 µL of **Taq polymerase** and 5 µl of **RT-Enzyme** in 145 µl of **Primers/probe mix**.

Reaction set-up

- Shake briefly the freshly prepared **PepMV-RT Master mix** and spin down the liquid.
- Add 19 µl of freshly prepared **PepMV-RT Master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
- Add 1 µl of RNA template to the **PepMV-RT 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	1 µl
Negative control	
PepMV-RT Master mix (14,5 µl of Primers/probe mix + 4 µl of Taq polymerase + 0,5 µl of RT-Enzyme)	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

- 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.
- 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	15 sec	
Annealing and elongation	60°C	60 sec	40

Results analysis

The reaction for *Pepino mosaic virus* will generate a specific FAM®-labeled amplification curve.

Fig.1: Example of an amplification curve relative to a PepMV positive sample.

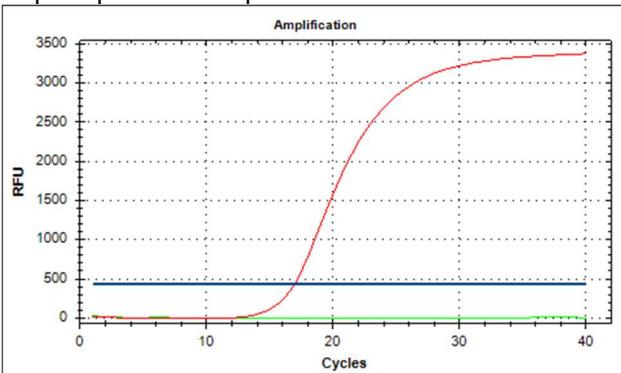


fig.1 shows the amplification curves associated to a PepMV-infected sample or **Positive Control** (red curve) and to a healthy sample or **Negative Control** (green curve)

ANALYSIS VALIDATION

The PCR plate is validated only when:

- the **Positive Control** generates an amplification curve for FAM® fluorophore. The Cycle threshold (Ct) value of the FAM®-labeled amplification curve should be below or equal to 37 (**fig.1**).
- the **Negative Control** does not generate any amplification curve associated to FAM® fluorophore or a curve is generated for FAM® fluorophore but the Ct is higher than 40 (**fig.1**).

RESULTS INTERPRETATION

When all the previous conditions are performed, the amplification results are interpreted as indicated in the **tab. 1**, by considering for each sample the Ct of the curve generated by FAM® fluorochrome specific to PepMV.

Ct	Interpretation
Ct ≤ 37	Positive
37 < Ct ≤ 40	Uninterpretable (*)
Ct > 40	Negative

tab.1 shows the results interpretation

(*) Test should be repeated

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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