Information pest: General potyvirus

The *Potyvirus* genus represents one of the largest plant virus genera containing more than 162 species. Potyviruses are one of the most economically important group of plant viruses and affect hundreds of crops.

Viruses within this genus are transmitted through aphid, seed, mechanical, graft and pollen.

Potyviruses can have a significant impact on crop production as loss of yield, unmarketable products and regulatory impact.

Introduction

The PCR PotyV set has been developed and optimized by Qualiplante according to Zheng et al., 2010 that is based on the amplification of NIb2F/NIb3R universal pair of primers designed on 2 conserved sites in the NIb (nuclear inclusion protein b) region. A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication.

RNA from 40 potyvirus isolates were tested. The NIb primers detected all the following potyviruses: *Apium virus Y, Bean common mosaic virus, Bean yellow mosaic virus, Carrot virus Y, Ceratobium mosaic virus, Clover yellow vein virus, Cowpea aphid-borne mosaic virus, Dasheen mosaic virus, Freesia mosaic virus, Johnsongrass mosaic virus, Papaya ringspot virus, Passion fruit woodiness virus, Peanut mottle virus, Potato virus Y, Sugarcane mosaic virus, Sweet potato feathery mottle virus, Turnip mosaic virus, Watermelon mosaic virus, Zucchini yellow mosaic virus, Wheat streak mosaic virus, Banana bract mosaic virus, Onion yellow dwarf virus, Sarcochilus virus Y* and *Wisteria vein mosaic virus.*
This product should be used only for research purposes.

Intended use

The PCR PotyV set is validated for the detection of general potyviruses in End-Point PCR.

Suitable samples are harvest fresh plant leaves tissues and tuber samples.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7PotyVP2	PCR General potyvirus (PotyV) set 24
7PotyVP9	PCR General potyvirus (PotyV) set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7PotyVP2-DM-	2x48 tests 7PotyVP9-DM-
Positive Control	3 tests 7PotyVP2-PC-	8 tests 7PotyVP9-PC-
Negative Control	3 tests 7PotyVP2-NC-	8 tests 7PotyVP9-NC-

Storage conditions

This set 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 set 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 cycler
- Agarose gel reagents and apparatus
- Reverse transcriptase enzyme

Nucleic acids extraction

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

Reverse transcribe the RNA extracted from your samples into complementary DNA (cDNA) according to your usual protocol.

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Reaction set-up

a) Slowly thaw Direct Master Mix by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and spin down the liquid.
c) Add 23 µl of Direct Master Mix (without cDNA template) to each PCR tubes or wells of an optical-grade PCR plate.
d) Add 2 µl of cDNA template to the Direct Master Mix. Do not forget to prepare a PCR tube or well of a PCR plate for the Positive Control and the Negative Control.

Components	Volume/PCR tube or well
cDNA template or Positive control or Negative control	2 µl
Direct Master Mix	23 µl
Total Volume / PCR tube or well	25 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 PCR thermal cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Initial denaturation	95°C	2 min	1
Denaturation	95°C	45 sec	35
Annealing	45°C	45 sec	
Elongation	72°C	45 sec	
Final elongation	72°C	5 min	1
Storage	4°C	∞	-

Agarose gel electrophoresis

Prepare an agarose gel **at 1,5% w/v in 1X-TAE 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 set)*.

Run: run the gel electrophoresis for 40-45 minutes at 80V.

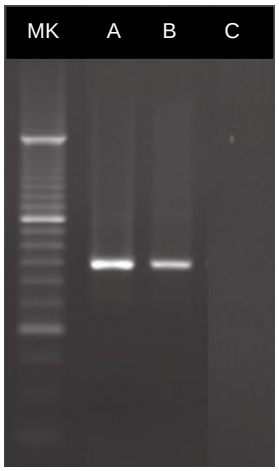
Results analysis

ANALYSIS VALIDATION

General potyvirus is detected when a 350 bp DNA fragment is observed.

The analysis is validated when:
- ✓ 1 DNA fragment of 350 bp is visible in the positive control lane.
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 1X-TAE 1,5% agarose gel showing the DNA amplification in a sample infected by Potato virus Y:



MK: DNA ladder (50-1'000 bp) - **A**: PVY positive sample or Positive Control of the set - **B**: PVY positive sample, dilution 1:10 - **C:** healthy sample or Negative Control of the set

RESULTS INTERPRETATION

The specific product of General potyvirus is a 350 bp fragment.

- ✓ A sample is **positive** when a 350 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 **350 bp**	Interpretation
-	NEGATIVE
✓	**POSITIVE** General potyvirus

POSITIVE: infected sample – NEGATIVE: healthy sample

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Special handling instructions

This set 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 set. Do not touch any set 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 sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. 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 set 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 correctly seal 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 any doubt, please, 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 set was not added into the reaction well	Repeat the test. If the problem persists, please, 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

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