Information pest: *Monosporascus cannonballus*

Monosporascus cannonballus is a root-infecting ascomycete fungus that infected mainly melons and watermelons. It has been reported on other members of *Cucurbitaceae* family. This pathogen is highly adapted to hot and dry areas and is often evenly distributed in fields resulting in devastating yield losses. This destructive disease is significant in the US, Israel, Spain and Japan and is spreading to new countries.

Characteristics symptoms of the disease are yellowing, death of the leaves and decline of the vines as plants approach maturity. A rapid collapse of the crop is typically observed just before harvest. Affected plants show root lesions, loss of secondary and tertiary roots, and in wet conditions, secondary root rot. This fungus causes root rot and necrosis which result in reduced growth, progressive defoliation and partial or complete collapse of the plants towards the end of the season.

Introduction

The PCR Monocano set has been developed by Qualiplante.

The primers pair was designed on the genome rDNA region of the Japanese strain of *Monosporascus cannonballus* (ITS1).
This product should be used only for research purposes.

Intended use

The PCR Monocano set is validated for the detection of *Monosporascus cannonballus* by End-Point PCR.

Suitable samples are roots from melons (*Cucurbita melo*) and watermelons (*Citrullus lanatus*) and fungal isolates.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7MnCn-P2	PCR *Monosporascus cannonballus* (Monocano) set 24
7MnCn-P9	PCR *Monosporascus cannonballus* (Monocano) set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7MnCn-P2-DM-	2x48 tests 7MnCn-P9-DM-
Positive Control	3 tests 7MnCn-P2-PC-	8 tests 7MnCn-P9-PC-
Negative Control	3 tests 7MnCn-P2-NC-	8 tests 7MnCn-P9-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)

- DNA 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

Nucleic acids extraction

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

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 DNA template) to each PCR tubes or wells of an optical-grade PCR plate.
d) Add 2 µl of DNA 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
DNA 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.

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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	30 sec	35
Annealing	60°C	30 sec	
Elongation	72°C	45 sec	
Final elongation	72°C	7 min	1
Storage	4°C	∞	-

Agarose gel electrophoresis

Prepare an agarose gel **at 2,5% 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.

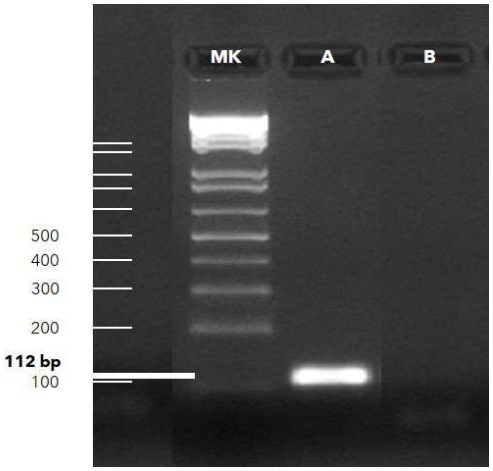
Results analysis

ANALYSIS VALIDATION

Monosporascus cannonballus is detected when a 112 bp fragment is observed.

The analysis is validated when:
- ✓ 1 DNA fragment of 112 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 2,5% agarose gel showing the DNA amplification in a sample infected by *Monosporascus cannonballus:*



MK: DNA ladder - **A**: Sample infected by *Monosporascus cannonballus* (112 bp) or Positive Control of the set- **B**: Healthy sample or Negative Control of the set

RESULTS INTERPRETATION

The specific product of *Monosporascus cannonballus* is a 112 bp fragment.

- ✓ A sample is **positive** when a 112 bp DNA specific fragment is present in the PCR reaction; in this case, the sample is infected by *Monosporascus cannonballus.*
- ✓ A sample is negative when no fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

Fragment size **112 bp**	Interpretation
-	NEGATIVE
✓	**POSITIVE** *Monosporascus cannonballus*

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

Qualiplante SAS guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Sets. 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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Sets components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change.

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