MGC
2024
v1

MEDICINAL GENOMICS®

PathoSEEK® Botrytis cinerea Detection Assay

QUICK GUIDE

Botrytis cinerea is a widespread necrotrophic plant pathogen that causes diseases on a plethora of plant species, including vegetables and ornamental greenhouse crops. On cannabis, the pathogen is responsible for causing the disease commonly known as bud rot, as well as seedling damping-off and leaf blight under certain conditions. The Botrytis cinerea Detection Assay targets the fungal organism *Botrytis cinerea*.

Test Kit Information

Assay Components:

1. PathoSEEK Botrytis cinerea qPCR Detection Assay - P/N 420116
    a. 1 tube (Store kit at -15 to -20°C). Expires 2 Years from Date of Manufacture.
2. PathoSEEK Botrytis cinerea qPCR Assay Positive Control - P/N 420222
    a. 1 tube (Store at -15 to -20°C). Expires 2 Years from Date of Manufacture.
3. Medicinal Genomics qPCR Master Kit v3 - P/N 420201
    a. Kit (Store at -15 to -20°C). Expires 2 Years from Date of Manufacture
    b. Reaction Buffer (10x)— 1 tube
    c. Nuclease Free Water—2 tubes
    d. qPCR Master Mix — 1 tube

Consumables:

1. 96-Well Optical qPCR plate - Medicinal Genomics P/N 100164
2. Adhesive optical seal for qPCR plates - Medicinal Genomics P/N 100177

DNA Lysis/Purification

For Plant Sampling and DNA lysis or purification instructions see the Sample Preparation Guide which should be followed *before* setting up the Botrytis cinerea Detection assay qPCR reactions.

qPCR Setup:

1. Prepare Assay Master Mix

qPCR Reagent Volumes

Reagents	1 Reaction
qPCR Master Mix	3.75 µL
Assay Probe Mix	1 µL
Reaction Buffer	0.8 µL
Water	8.2 µL
Total Assay Probe MM	**13.75 µL**

   a. Prepare enough master mix for your samples plus two controls (positive and NTC). Add 10% overage to the master mix components to account for pipetting and dead volumes.

2. Prepare Positive Control Dilution
   a. Dilute the stock assay positive control 1:10 with nuclease free water. 9 µL water, 1 µL positive control, vortex and spin down.
3. Transfer samples and master mix to PCR plate
   a. Transfer 5 µL of each sample, 5 µL of diluted assay positive control and 5 µL of water to separate wells of a qPCR plate
   b. Transfer 13.75 µL of freshly prepared qPCR Assay Master Mix to each well and slowly tip mix. Avoid adding bubbles to the mixture.
4. Seal plate, spin in plate centrifuge and load on qPCR instrument.
5. Botrytis cinerea - qPCR cycling parameters:
   a. Hot Start at 95 ºC for 5 minutes
      Followed by 40 cycles of:
   b. 95 ºC for 15 seconds
   c. 65 ºC for 1 min and 30 seconds.
   d. Plate Read

6. Start the run.
7. When the run is complete, the plate can be discarded.
8. Proceed to data analysis.

Data Analysis:

Botrytis cinerea Assay	Cq Value	Fluor	Negative Control (Cq)	Cq Threshold
Positive *Botrytis cinerea* Result	< 40	FAM	No Value	Presence/Absence
Internal Cannabis Control*	<35	HEX	*Internal control verifies the presence or absence of cannabis DNA	
Assay Positive Control	<35	FAM		

1. Positive and No Template (NTC) Controls - Confirm Assay Positive control well and assay NTC well results are as expected.
    a. Assay positive control should have a Cq value ≤ 35 for FAM.
        i. No HEX signal should be observed in the control wells
            1. If HEX signal is observed a Cq of >35 is acceptable.
    b. Assay NTC should have no Cq value for FAM.
        i. No HEX signal should be observed in the control wells
            1. If HEX signal is observed a Cq of >35 is acceptable.
    c. Confirm Cq values against amplification plots.
2. Sample Analysis
    a. Internal Cannabis Control (HEX)
        i. HEX signals in sample wells should be ≤ 35
    b. Botrytis cinerea Positive samples (FAM)
        i. FAM amplification which results in a Cq value ≤ 40
    c. Confirm Cq values against amplification plots.

REVISION HISTORY

Version	Date	Description
v1	November 2024	Quick Guide Format

DISCLAIMER

This test was developed, and its performance characteristics determined by Medicinal Genomics Company, for laboratory use. Any deviations from this protocol are not supported by MGC. The results may vary based on laboratory conditions. All thresholds were determined based on the results using the Agilent AriaMX or BIO-RAD CFX96 Touch® Real-Time PCR Detection System.

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