



PathoSEEK®

Environmental Screening

with SenSATIVax® DNA Purification

QUICK GUIDE

Introduction

Plant pathogens can infect cannabis crops at the roots, crown, and foliage, reducing their ability to grow. Molds can also colonize the cannabis flower during development or after harvest, which can compromise quality and pose a health risk to consumers. Now, cannabis cultivators and processors can use the same method the top cannabis testing labs use to detect microbial contamination on end products to screen their environment and equipment.

The following SOP describes how to collect samples from facilities and equipment that can be tested for microbial contamination using the SenSATIVAx® DNA Purification Kit and PathoSEEK Microbial Safety Testing Platform.

Test Kit Information

Assay Components:

1. SenSATIVAx® Flower & Leaf DNA Purification Kit — P/N 420001 (200 extractions).
 - a. MGC Lysis Buffer.— 1 bottle. Store at 20-28°C. Expires 1 Year from DOM
 - b. MGC Binding Buffer.—1 bottle. Store at 2-8°C. Expires 1 Year from DOM
 - c. MGC Elution Buffer.—1 bottle. Store at 20-28°C. Expires 1 Year from DOM
2. SCCG Control (referred to as ICC in this user guide) - P/N 420326. (60 ul)
 - a. Control - 1 tube. Store at -15 to -20°C. Expires 2 Years from DOM
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
4. Nuclease Free Water, Medicinal Genomics P/N 420184
5. PathoSEEK® **Organism of interest** qPCR Detection Assay - P/N
 - a. 1 tube (Store kit at -15 to -20°C). Expires 2 Years from Date of Manufacture.
6. PathoSEEK® **Organism of interest** qPCR Assay Positive Control - P/N
 - a. 1 tube (Store at -15 to -20°C). Expires 2 Years from Date of Manufacture.

Consumables & Hardware:

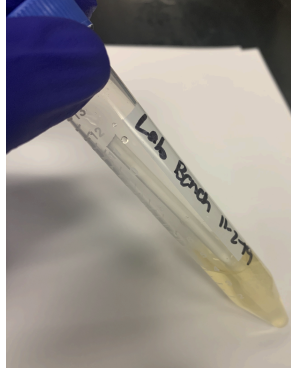
- Puritan Sterile Foam Tipped Swabs (Puritan, SKU#: 25-1506)
- 1.5 mL Eppendorf tubes (Multiple Suppliers)
- Scissors
- 15mL Conical Tubes (USA scientific, # 1475-0511)
- Single channel pipettes P20, P200, & P1000
- Filtered pipette tips for P20, P50, P200, & P1000
- Eppendorf tube rack
- Incubator, that can reach 37 °C (VWR® Personal Size Incubator # 97025-630, or similar)

Swabbing Instructions:

1. Remove the MGC Enrichment Broth from the 2-8°C refrigerator
2. Inoculate enrichment broth with sample swab
 - a. Label a 15 mL tube with “[swab area] [date]”.
 - b. Add 3 mLs of MGC Enrichment Broth to the 15 mL tube.

Note: MGC Enrichment Broth is a growth medium and the perfect condition for microbes to grow. Due to this, it is best to pour the approximate amount of MGC Enrichment Broth into another sterile tube or container so as to not contaminate the whole bottle. Nothing should go into this bottle. Return it to the 2-8°C refrigerator immediately after use.

- c. Remove Puritan swab from wrapper and cut in half so it will fit into the 15 mL conical tube.
- d. Moisten swab with nuclease free water.
- e. Swab area of interest and place swab into TSB inside 15 mL tube.



- f. Loosely screw lid on 15 mL tube and place in 37 °C incubator for 16 - 48 hours.

DNA Extraction

1. Prepare the Internal Cannabis Control (ICC) control dilution of 1:5,000
 - a. Label a new 1.5 mL Eppendorf tube (ICC 1:50). Add 1 μ L of ICC positive control into 49 μ L of dH₂O. Vortex to mix thoroughly and quick spin tube.
 - b. Label another 1.5 mL Eppendorf tube (ICC 1:5,000). Add 99 μ L of dH₂O, then add 1 μ L of the ICC 1:50 dilution. Vortex to mix thoroughly and quick spin tube.
This will result in a 1:5,000 dilution of ICC.
2. Remove 15 mL tubes from the incubator.
3. Transfer 1 mL enriched sample from each 15 mL tube into a 1.5 mL tube.
4. Add 50 μ L of MGC Lysis buffer and vortex for 10 seconds then let incubate on the bench for 2 minutes.
5. Pellet cellular debris by spinning in a high-speed centrifuge for 1-3 minutes. Note: The supernatant should be translucent at this point. If the sample is still opaque (cloudy) spin for longer. This is important for removing cellular debris.
6. Transfer 200 μ L of supernatant to a labeled 96-well extraction plate. Label the extraction plate with the [date].
Note: Do not disturb the pellet in the 1.5ml tube, the pellet size will vary depending on trichome density.
7. Vortex MGC Binding Buffer thoroughly before use, be sure that the magnetic particles are completely re-suspended in the buffer for at least 30 seconds.
8. Add 200 μ L of MGC Binding Buffer to each sample, and pipette tip mix 15 times.

Note: Be careful to avoid adding too many bubbles by pipetting gently when tip mixing. This is extremely important as to not contaminate the wells in proximity.

9. Incubate the plate on the bench for at least 5 minutes.
10. Place the extraction plate onto the 96-well plate magnet plate for at least 5 minutes.
11. After the 5 minute incubation, remove as much of the 400 μ L of the supernatant as possible. Be careful not to disturb or aspirate the beads.
 - a. Add 400 μ L of 70% ethanol (EtOH) with the extraction plate still on the magnet plate.
 - b. Wait at least 30 seconds and remove all the EtOH. Note: Place the pipette tip at the bottom center of the well to remove all liquid.
12. Repeat 400 μ L 70% EtOH wash with the extraction plate still on the magnet plate. Wait at least 30 seconds and remove all the EtOH.

Note: If EtOH still remains in the wells, go back in with a smaller pipette tip to remove the excess. Leftover EtOH can inhibit qPCR efficiency.
13. After all the EtOH has been removed, allow the beads to dry for up to 15 minutes. Necessary drying time will vary based on the complete removal of the second ethanol wash, as well as the lab environment. Visually inspect beads for residual ethanol before the elution step.

Note: It is important to NOT allow the beads to dry for an extended period of time. Over-drying can cause a reduction in DNA yield.
14. Remove the extraction plate from the magnet plate and add 50 μ L of MGC Elution Buffer.
 - a. Tip mix approximately 15 times or until the beads are completely re-suspended.

Note: The re-suspensions may appear varied in their appearance, but the result will be the same.
 - b. Incubate the plate for at least 1 minute on the bench, before returning the plate to the magnetic plate.
 - c. Let the plate sit on the magnet for at least 1 minute before transferring the eluent to a new extraction plate labeled with “Final Extract [date]”.
15. Seal the plate with the adhesive seal, making sure to completely seal the plate wells. Slide a pen or flat object back and forth along the seal. Store at -20 °C until ready to perform the qPCR protocol.

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 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 with sample. Avoid adding bubbles to the mixture.
4. Seal plate, spin in plate centrifuge and load on qPCR instrument.
5. Set up Assay - qPCR cycling parameters: **These may vary depending on organism of Choice - confirm cycling conditions in assay specific user guide**
 - a. Hot Start at 95°C for 5 minutes
Followed by 40 cycles of:
 - b. 95°C for 15 seconds
 - c. **65°C** for 90 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:

MGC Detection Assay	Cq Value	Fluor	Negative Control (Cq)	Cq Threshold
Positive Detection Assay Result	< 35	Varies based on assay used	No Value	Presence/Absence
Internal Cannabis Control*	<35	HEX	*Internal control verifies the presence or absence of cannabis DNA	
Assay Positive Control	<35	Varies based on assay used		

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 .
 - 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.
 - i. No amplification of the target of interest should be observed in the NTC
 - ii. 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. Organism Positive samples (varies based on assay used)
 - i. Amplification which results in a Cq value ≤ 35
 - c. Confirm Cq values against amplification plots.

REVISION HISTORY

Version	Date	Description
v2	March 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.

LIMITED USE LABEL LICENSE

This product is covered by at least one or more claims of US patent applications, which are exclusively licensed to Medicinal Genomics Corporation. This product is sold strictly for the use of the buyer, and the buyer is not authorized to transfer this product [or any materials made using this product] to any third party.

© 2023 Medicinal Genomics Corporation. All rights reserved.

* All Trademarks are property of their respective owners.