MGC
2023

MEDICINAL GENOMICS®

PathoSEEK®

5-Color Aspergillus Multiplex Assay

with SenSATIVAx® DNA Purification

QUICK GUIDE

QUICK GUIDE: PATHOSEEK 5 COLOR ASPERGILLUS DETECTION ASSAY

DNA Purification:

Flower:

1. Weigh 1g sample into one side of the mesh lining of a Whirl-Pak bag then transfer 9 mL TSB into the bag. Hand homogenize for 1 minute.
2. Enrich at 37°C 24-48 hours.
3. Remove from the incubator and hand homogenize for 1 minute.
4. Transfer 1mL from the bag (non sample side of mesh lining) into a 1.5 mL tube.
5. Add 50 µL MGC Cell Lysis Buffer.
6. Vortex for 10 seconds. Let sit for 2 minutes then centrifuge at high speed for 5 minutes.
7. Transfer 200 µL supernatant to a well of extraction plate. Avoid pellet at bottom of tube.
8. Add 200 µL MGC DNA Binding Buffer and tip mix. Let sit at room temp 5 minutes.
9. Move the extraction plate to the magnet. Let the beads settle for 5 minutes.
10. Remove and discard supernatant (~400 µL) while avoiding beads.
11. Add 400 µL 70% Ethanol. Wait 30 seconds. Remove and repeat for a total of 2 washes.
12. Let the beads dry for 5-15 minutes.
13. Remove plate from magnet, add 50 µL MGC Elution buffer and mix to resuspend beads. Let sit for one minute.
14. Move the plate to the magnet.
15. Let beads settle for 1 minute, then transfer the eluent to a new well or plate.

Non-Flower:

1. Weigh 1g sample into 15 or 50 mL tube.
2. Add 2.4 mL TSB, vortex.
3. Enrich at 37ºC for 24-48 hours.
4. Add 4.6 SenSATIVAx MIP Solution A.
5. Vortex well to adequately homogenize.
6. Transfer 1 mL to 1.5 mL tube.
7. Add 10 µL of a Fresh 1:5k dilution of SCCG Internal Control.
8. Vortex and spin for 10 minutes in high-speed centrifuge.
9. Transfer 600 µL to a new tube.
10. Add 600 µL chloroform.
11. Vortex well and centrifuge 5 minutes in high speed centrifuge.
12. Transfer 100 µL of supernatant to a well of extraction plate.
13. Add 100 µL SenSATIVAx MIP Solution B and tip mix.
14. Add 200 µL MGC DNA Binding Buffer and tip mix, let sit at room temp 5 minutes.
15. Move plate to magnet, let beads settle for 5 minutes.
16. Remove and discard supernatant (~400 µL) while avoiding beads.
17. Add 400 µL 70% Ethanol, wait 30 seconds, remove, repeat for a total of 2 washes.
18. Let the beads dry for 5-15 minutes.
19. Remove plate from magnet, add 50 µL MGC Elution buffer, resuspend bead ring.
20. Let sit for 1 minute, move the plate to the magnet.
21. Let beads settle for 1 minute, transfer eluent to a new well.

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qPCR Setup:

1. Prepare Master Mix and Positive Control Dilution

   PCR 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

2. 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.
3. Dilute the stock assay positive control 1:10 with nuclease free water. 9 µL water, 1 µL positive control, vortex and spin down.
4. 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.
5. Transfer 13.75 µL of freshly prepared qPCR Master Mix to each well and slowly tip mix.
6. Seal plate, spin in plate centrifuge and load on qPCR instrument.
7. Set up MGC qPCR cycling parameters:
   a. 95°C, 5 minutes
   b. 40 cycles of:
      i. 95°C, 15 seconds
      ii. 65°C, 90 seconds
8. Start run - Run takes approximately 1 hour and 40 minutes.

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Data Analysis:

PathoSEEK™ Assay	Cq Value	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
Aspergillus niger	≤ 40	ROX	No Cq	Presence/Absence
Aspergillus flavus	≤ 40	Cy5	No Cq	Presence/Absence
Aspergillus fumigatus	≤ 40	FAM	No Cq	Presence/Absence
Aspergillus terreus	≤ 40	Cy5.5 (Bio-Rad) or ATTO 425 (Aria MX)	No Cq	Presence/Absence
Internal Control*	≤35	HEX	*Internal control verifies the presence or absence of plant DNA obtained through the DNA Purification Process	
Assay Positive Control	≤35	FAM/ROX/Cy5/Cy5.5 or ATTO 425		

1. Confirm Assay Positive control well and assay NTC well to ensure the results are as expected.
    a. Assay positive control should have a Cq value ≤ 35 for FAM, Cy5, ROX, Cy5.5/Atto425
        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, Cy5, ROX and Cy5.5/Atto425
        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. Unknown Samples
    a. Internal DNA Purification Control (HEX)
        i. HEX signals in sample wells should be ≤ 35 for flower ≤ 40 for non flower
    b. Samples positive for Aspergillus will show amplification which results in a Cq value ≤ 40
        i. See table above to determine which species of aspergillus is present
    c. Confirm Cq values against amplification plots

For detailed User Guide please visit the Medicinal Genomics website https://medicinalgenomics.com/product-literature/

Workflow Diagram: SenSATIVAx DNA Purification from Flower

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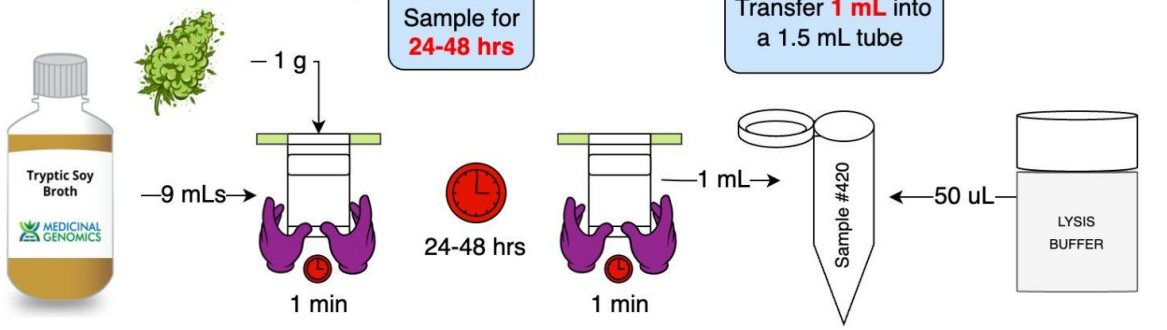
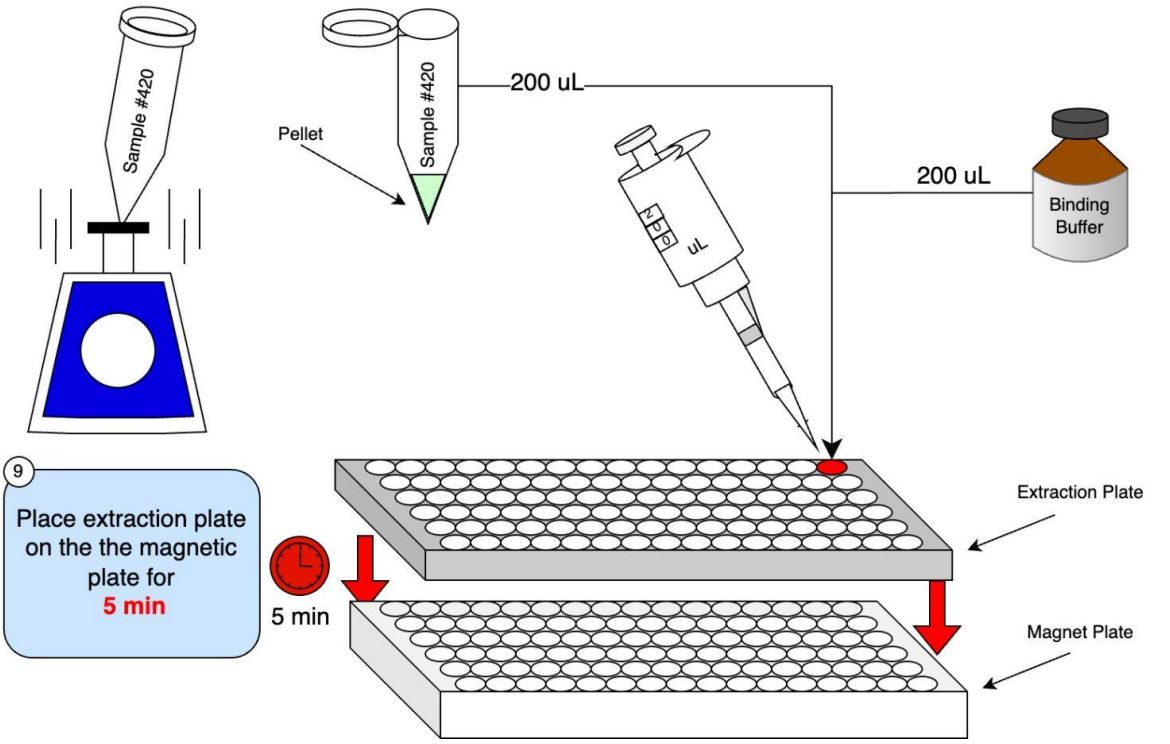
SenSATIVAx®
DNA Purification from FLOWER
Aspergillus Assay

1. Transfer **9 mL** of Tryptic Soy Broth and 1g of sample into WP bag. Hand homogenize for **1 min**
2. Enrich Sample for **24-48 hrs**
3. Hand homogenize for **1 min**
4. Transfer **1 mL** into a 1.5 mL tube
5. Add **50 uL** of Lysis Buffer to Sample Tube



6. Vortex **10 sec** and incubate at room temp for **~2 min**. Centrifuge to pellet debris
7. Add **200 uL** of the sample supernatant to the a well on extraction plate
8. Add **200 uL** binding buffer and tip mix. Let sit at room temp for **5 min**



9. Place extraction plate on the the magnetic plate for **5 min**

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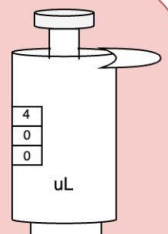
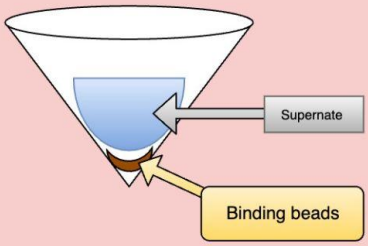
STEPS 10-12
KEEP ON MAGNETIC PLATE

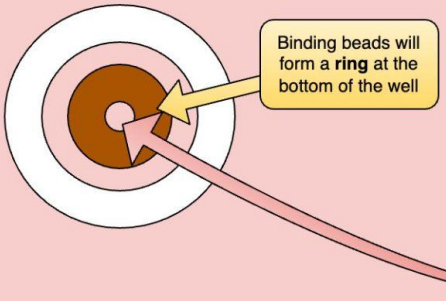
10 Pipette to the bottom center of the plate well **WITHOUT** disturbing the ring of binding beads Remove and discard all of the supernatant



Side view of well



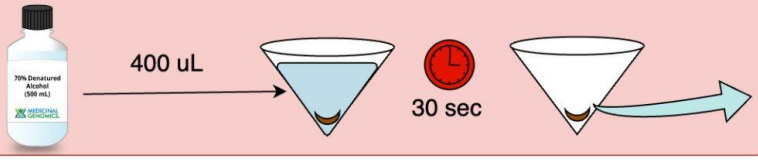
Top view of well



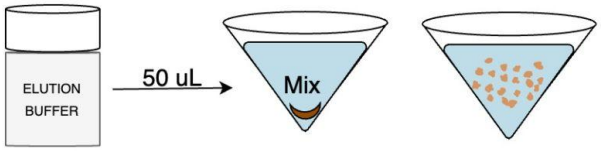
11 Pipette **400 uL** of 70% Ethanol into well (DO NOT MIX). Let it sit for **30 seconds**, then remove all ethanol **WITHOUT** disturbing the ring of binding beads.

Repeat this step! (2 washes)

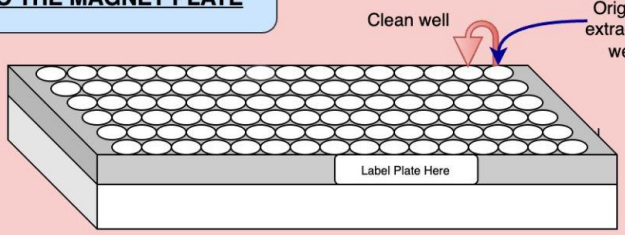
12 Let binding beads dry for **5-15 minutes** Make sure they do not over dry!



13 **REMOVE FROM MAGNET PLATE**
Add **50 uL** of Elution Buffer to each well and mix until the binding beads are fully resuspended.
Let sit for **1 minute**



14 **PLACE EXTRACTION PLATE ONTO THE MAGNET PLATE**



15 Let beads settle for **1 minute**, then transfer **50 uL** the eluent to a new clean well

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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. Altitude and humidity are among factors known to affect the growth of bacterial and fungal species. 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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