



FemINDICator® Cannabis qPCR Sex Detection Assay

QUICK GUIDE v3

Materials

FemINDICator Cannabis Sex Detection Assay Kit v3 - P/N 420546 (**200 reactions**)

Component Name	Qty Provided	Storage Conditions
PathoSEEK® Amplification Mix <i>Includes 2 tubes nuclease free water for resuspension</i>	4 Vials (50 rxns/each)	RT / -20 °C*
FemINDICator Cannabis Sex Detection Assay v3	1 Tube (200 µL)	-20 °C

Note: Actual fill volumes include overage

*The PathoSEEK Amplification Mix can be stored lyophilized at Room Temperature for up to 2 years. Once re-hydrated it must be stored at -20 °C for up to 3 months.

Additional Required Components Not in Kit:

Item P/N	Item Name	Qty Provided	Storage Conditions
420240	Quick Lysis*	12 x 0.2 mL PCR 8-tube strips in a 96 well carrier	RT (20–28 °C)
420312	FemINDICator Positive Control	1 Tube (50 µL)	-20 °C
100184	PCR Grade Water	1 Bottle (500 mL)	RT
100177	Optical Adhesive Seals	Case of 100	RT
100164	96-Well Optical qPCR plate	Case of 25	RT

*4mm Grommets for leaf punches are included with Quick Lysis reagent (Qty 100)

DNA Lysis

For Plant Sampling and DNA lysis instructions, see the [Sample Preparation Guide](#), which should be followed *before* setting up the FemINDICator qPCR.

qPCR Setup:

1. Prepare Assay Master Mix

qPCR Reagent Volumes

Reagent	1 Reaction
PathoSEEK Amplification Mix	10 µL
FemINDICator Assay v3	1 µL
Water	4 µL
Total Assay Probe MM	15 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. Positive Control Dilutions
 - a. Standard qPCR Instruments (Bio-Rad CFX96 and Agilent AriaMX)
 - i. Prepare a 1:10 dilution from the stock
 1. After the positive control stock tube is fully thawed, vortex and quickly spin prior to use.
 2. Add 2 µL of stock positive control to 18 µL nuclease free water (found in the kit) and vortex to mix. Quick spin the tube after mixing. This is a 1:10 dilution.
 - b. BMS Mic
 - i. Prepare a 1:100,000 dilution from stock
 1. After the positive control stock tube is fully thawed, vortex and quickly spin prior to use.
 2. Add 2 µL of positive control to 198 µL nuclease free water (found in the kit) and vortex to mix then quick spin the tube. This is a 1:100 dilution.

3. Add 2 µL of the 1:100 positive control dilution to 198 µL nuclease free water and vortex to mix then quick spin the tube. This is a 1:10,000 dilution.
4. Add 10 µL of 1:10,000 FemINDICATOR Positive Control to 90 µL of nuclease-free water. Vortex and pulse spin down in a mini centrifuge. This is the 1:100,000 dilution.

Note: It is best to add the largest volume reagent first, in this case the 198 µL water then the 2 µL of positive control, pipette mix or vortex control dilution to ensure control DNA is in solution. This dilution is stable when it is stored at -20°C when not in use for six months.

3. Transfer samples and master mix to the 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 15 µL of freshly prepared qPCR Assay Master Mix to each well and slowly tip mix 5 times. Avoid adding bubbles to the mixture.
4. Seal plate, spin in plate centrifuge, and load on qPCR instrument.
5. Set up FemINDICATOR qPCR cycling parameters:
 - 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:

Assay	Cq Value	Fluor	Negative Control (Cq)	Cq threshold
Sex - Male	≤ 35	FAM	No Value	Presence/Absence
Sex - Female	> 35	FAM	No Value	Presence/Absence

Assay	Cq Value	Fluor	Negative Control (Cq)	Cq threshold
Internal Control*	≤ 35	HEX	*Internal control verifies the presence or absence of cannabis DNA	
Assay Positive Control	≤ 35	FAM		

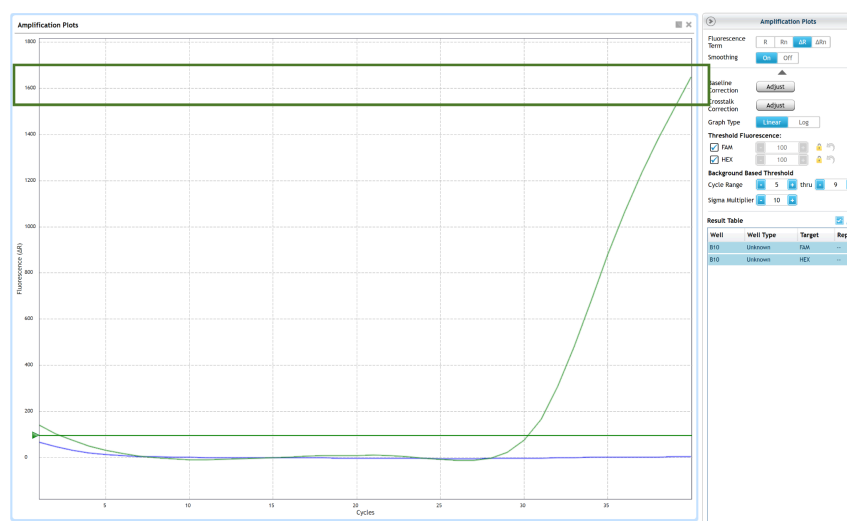
1. Positive and No Template (NTC) Controls - Confirm Assay Positive control well and assay NTC well results are as expected.

- a. The assay positive control should have a Cq value ≤ 35 for FAM.
 - i. No HEX signal should be observed in the control wells.
 1. If a HEX signal is observed, a Cq of >35 is acceptable.
- b. Assay NTC should have no Cq value for FAM.
 - i. A FAM Cq value in the NTC is not acceptable.
 - ii. No HEX signal should be observed in the control wells.
 1. If a HEX signal is observed, a Cq of > 35 is acceptable.
- c. Confirm Cq values against amplification plots.

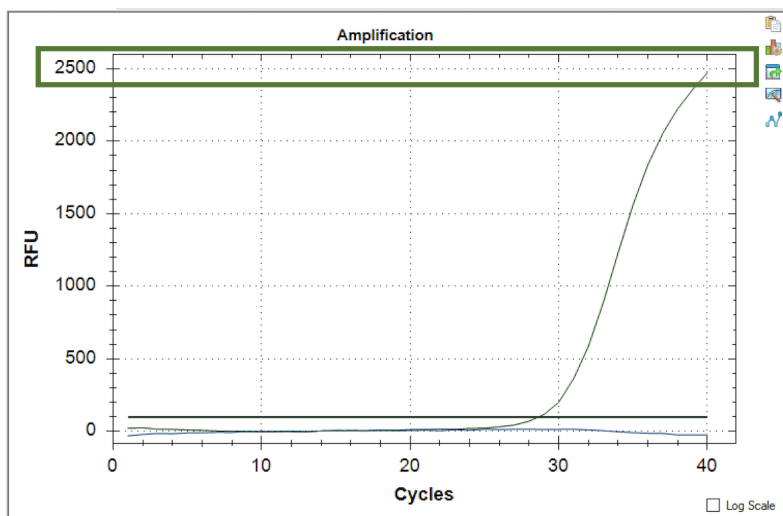
2. Sample Analysis

- a. Internal Control (HEX) should be present for both male and female results.
 - i. HEX Cq values in all sample wells should be ≤ 35 and the amplitude should be greater than 500 RFU.
 1. For the Agilent AriaMX and Biorad CFX96, RFU may be found in the software analyzed view of the chosen software using the linear scale.

a. AriaMX - ΔR - Female Result

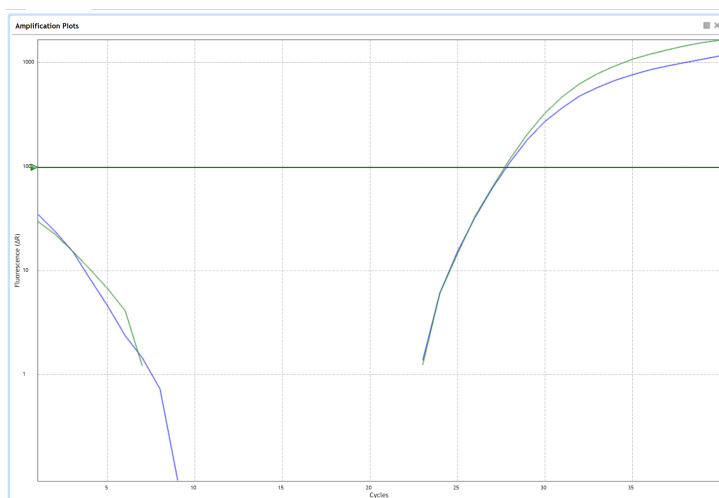


b. Biorad CFX96 - Baseline Subtracted Curve Fit - Female result



b. Male samples (FAM)

- i. FAM amplification which results in a Cq value ≤ 35
- ii. Example of a male result:



c. Confirm Cq values against amplification plots.

Troubleshooting Guide

Symptom	Reason	Solution
Internal control failure	Nucleic Acid Isolation Failure	Repeat Quick Lysis or PurePrep by following the protocol.
	If using PurePrep Residual ethanol in elution	Ethanol is an inhibitor to RT-qPCR. Return to the PurePrep protocol and repeat all steps.
	qPCR inhibition	Dilute extracted or lysed samples 1:10 with qPCR grade water and repeat the RT-qPCR.
	Mix up in Reaction Setup	Repeat the RT-qPCR by following the protocol.
	Missing Fluorophore on plate set up	Check Plate Setup to ensure the correct fluorophores were chosen on setup of run. They can be corrected post run.
Amplification of the Internal control is not expected in the assay positive or negative control wells. No Cq or a Cq of more than 35 is acceptable. Any Cq lower than 35 constitutes a rerun.	Plant DNA contamination in a reagent	Troubleshoot which reagent was contaminated. Use new reagents. Thoroughly clean all pipettes and bench areas with 10% bleach solution.
	qPCR bench too close to extraction area	Designate separate benches, pipettes etc. for extractions and RT-qPCR setup
Positive Negative Control	Small Cq value <15	Visually confirm that there is a true amplification curve. If not, this may be considered a background trace.
	Contamination	Repeat the RT-qPCR by following the protocol.
Positive Negative Control (Continued)	Insufficient pre-setup bleaching	Clean workspace and all equipment with 10% Bleach, repeat rt-qPCR.
Negative Positive Control	Mix-up in Reaction Setup	Repeat the RT-qPCR by following the protocol.
Total run failure	Excessive vortexing the RT-qPCR Master Mix	Repeat the RT-qPCR by following the protocol.
Background Amplification	Unclear	This is usually seen with a very low Cq reading (<15), the curve is usually missing the exponential growth phase, but rather displays a gradual increase of fluorescence signal. This is usually a negative result, but should be repeated.

REVISION HISTORY

Version	Date	Description
v1	November 2023	Quick Guide Format
v2	June 2025	• Optimal Positive Control dilution update for MIC • HEX amplitude requirement in data analysis • Addition of previous master mix volumes and cycling conditions for difficult samples
v3	November 2025	• Updating qPCR Master Mix v3 to Amplification Mix • Update to Kit format • Removed Master Mix volume and cycling options - reverted back to original optimal parameters

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 Biomolecular Systems MIC, Agilent AriaMX and BIO-RAD CFX96 Touch® Real-Time PCR Detection System.

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