



PathoSEEK[®] Hop Latent Viroid Detection Assay v2

QUICK GUIDE

Test Kit Information

Assay Components:

1. PathoSEEK® RT-qPCR Master Kit Version 2 - P/N 420207
2. PathoSEEK® Hop Latent Viroid Positive Control - P/N 420123
3. PathoSEEK® Hop Latent Viroid Detection Assay Version 2 - P/N 420135

Consumables:

1. 96-Well Optical qPCR plate - Medicinal Genomics P/N 100164
2. Adhesive optical seal for qPCR plates - Medicinal Genomics P/N 100177
3. PCR Grade Nuclease Free Water - Medicinal Genomics P/N 420184

Nucleic Acid Lysis/Purification

For Plant Sampling and nucleic acid extraction or purification see the Sample Preparation Guide which should be followed *before* setting up the HLVD RT-qPCR.

RT - qPCR Setup:

1. To rehydrate the RT-qPCR Master Kit v2 add 675ul of Nuclease Free water and swirl or gently pipette tip-mix. Store at -20 °C when not in use. Unused resuspended master mix can be stored at -20°C for up to 3 months or at 4°C for up to 3 weeks.
2. Prepare Assay Master Mix

RT- qPCR Reagent Volumes

Reagents	1 Reaction
RT - qPCR Master Kit v2 (rehydrated in 675 µL H ₂ O)	10 µL
HLVd Assay Probe Mix	1 µL
Total Volume	11 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.
3. 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.
4. Transfer samples and master mix to PCR plate
 - a. Transfer 11 µL of freshly prepared RT-qPCR Assay Master Mix to each well.
 - b. Transfer 9 µL of each sample, 9 µL of diluted assay positive control and 9 µL of water to separate wells of a qPCR plate and slowly tip mix. Avoid adding bubbles to the mixture.
5. Seal plate, spin in plate centrifuge and load on qPCR instrument.

6. Set up HLVD RT-qPCR cycling parameters:
 - a. 25 °C for 30 seconds.
 - b. 55 °C for 10 minutes
 - c. 95 °C for 1 minute
Followed by **40** cycles of:
 - d. 95 °C, 10 seconds
 - e. 65 °C, 30 seconds
 - f. Plate read
7. Start the run.
8. When the run is complete, the plate can be discarded.
9. Proceed to data analysis.

Data Analysis:

PathoSEEK Assay	Cq Value	Fluor	Negative Control (Cq)	CFU threshold (CFU/g)
<i>HLVd</i>	≤ 35	FAM	No Cq	Presence/Absence
Internal Cannabis Control*	≤35	HEX	*Internal cannabis control verifies the presence or absence of cannabis DNA obtained through the nucleic acid (DNA/RNA) Purification Process	
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. Cq values >35 in FAM signal are acceptable
 - 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. HLVD positive samples (FAM)
 - i. FAM amplification which results in a Cq value ≤ 35
 - 1. If the Cq value is between 35 and 40, re-test the plant from a fresh root sample. If the value goes below 35 the sample should be considered positive for HLVD. If the Cq value is still between 35 and 40 the result is inconclusive.
- c. Confirm Cq values against amplification plots.

REVISION HISTORY

Version	Date	Description
v1	November 2023	Quick Guide Format and Product Launch

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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