MGC
2025
v3

PathoSEEK® Hop Latent Viroid Detection Assay v2

QUICK GUIDE v3

Test Kit Information

Assay Components:

1. PathoSEEK® Hop Latent Viroid Detection Assay v2 **Kit**- P/N 420512
    a. Assay — 1 tube (Store at -15 to -20 °C). Expires 2 Years from Date of Manufacture.
    b. PathoSEEK® Amplification Mix - 4 Vials - store lyophilized at room temperature until ready to use. Before first use rehydrate in 550 µL of Nuclease Free Water and swirl or gently tip mix. Unused resuspended master mix can be stored at -20°C for up to 3 months.
2. PathoSEEK® Hop Latent Viroid Positive Control - P/N 420123

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.

Questions? Contact Customer Support at support@medicinalgenomics.com or 866.574.3582

RT - qPCR Setup:

1. To rehydrate the PathoSEEK® Amplification Mix add 550 µL of Nuclease Free water and swirl or gently pipette tip-mix. Store at -20 °C when not in use. Unused resuspended Amplification Mix can be stored at -20 °C for up to 3 months.

2. Prepare Assay Master Mix

   RT- qPCR Reagent Volumes

Reagents	1 Reaction
PathoSEEK® Amplification Mix (rehydrated in 550 µL H_2O)	10 µL
HLVd Detection Assay	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, controls, 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 ºC 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)
HLVd	≤ 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. Internal Cannabis Control, on the HEX fluorophore, has a Cq value ≤ 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.

Troubleshooting Guide:

Symptom	Reason	Solution
Internal cannabis control (ICC Primer) 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.
Internal Cannabis Control (ICC) Positive result on positive or negative control samples or samples that do not contain plant DNA 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.

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REVISION HISTORY

Version	Date	Description
v1	November 2023	Quick Guide Format and Product Launch
v2	January 2025	1. Name change - RT-qPCR Master Mix v2 changing to PathoSEEK® Amplification Mix. Additional vial included - 3 total for 200 reactions 2. HLVd v2 Assay sold as kit with Amplification Mix 3. Added Troubleshooting Guide
v3	February 2025	1. Updating Amplification Mix to new format (4 vials @ 50 reactions each vial) 2. Introduction of kit format - Assay and Amplification Mix sold together

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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