SIGNALCHEM DIAGNOSTICS

190-13160 Vanier Pl, Richmond,
B. C., Canada, V6V 2J2
+ 1 778 326 0223
info@signalchemdx.com
www.signalchemdx.com

INSTRUCTIONS FOR USE

HLVd Cannabis qPCR Detection Kit — HLV01-K198

Intended Use

For detection of Hop Latent Viroid in Cannabis samples using RT-qPCR.

Introduction

The HLVd Cannabis RT-PCR Detection Kit is designed to detect Hop Latent Viroid (HLVd) in cannabis samples using one-step real-time PCR to analyze extracted RNA. The kit also includes a cannabis housekeeping gene as a control, which helps monitor PCR inhibition and verify the quality of the input RNA. This ensures reliable and straightforward detection of HLVd in cannabis samples.

Background

Hop Latent Viroid (HLVd) significantly impacts cannabis by stunting plant growth, reducing yields, and impairing bud quality. Infected plants often exhibit symptoms like leaf curling and discoloration, and their overall vigor is diminished. For commercial growers, the economic effects can be severe, with decreased harvests and increased costs associated with disease management and prevention. HLVd spreads primarily through contaminated plant material, making stringent sanitary practices and using disease-free plants crucial for managing its impact.

Disclosure

This product is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in therapeutic, consumer, or pesticidal applications.

This product has been optimized for use on the Bio-Rad CFX Opus 96 Real-Time PCR System. Use with other comparable systems may require additional validation to ensure diagnostic accuracy.

Kit Components (for 100 reactions)

No	Component #	Component	Amount
1	HLV-A	5X Reaction Mix	480 µL
2	HLV-B	Enzyme Mix	120 µL
3	HLV-C	Primer-Probe Mix	120 µL
4	CPT-E	Nuclease-free Water	1.5 mL
5	HLV-D	HLVd Positive Control	50 µL

Not Provided

- Real-Time PCR system with FAM and HEX filter channels
- RNA Extraction and Purification Kit
- Gloves

- Microcentrifuge
- Microcentrifuge tubes and PCR tubes
- Vortexer
- Micropipettors
- Sterile pipette filter tips

Kit Storage

All kit components should be stored frozen (-20°C).

Before use, allow the kit components to completely thaw and vortex to ensure no precipitation.

Protect primer-probe mix from direct light.

Precautions

- Ensure a clean working environment before experiment.
- Separate the specimen extraction, reaction setup, and amplification/detection processes in different facilities to prevent cross-contamination including equipment and supplies.
- Presence of PCR inhibitors may cause invalid or false negative results.
- Possible mutations in the target regions of the HLVd genome that are covered by the primers in this kit could lead to the inability to detect the pathogen.
- Following good laboratory practices is essential for the proper use of this kit. Ensure that both the kit and the reactions remain free from contamination. Carefully inspect all reagents for any signs of contamination and do not use any reagents that appear to be contaminated.

Protocol

1. Prepare the reactions by combining the following components:

Component	Volume
Nuclease-free Water (4)	12 µL
5X Reaction Mix (1)	4 µL
Enzyme Mix (2)	1 µL
Primer-Probe Mix (3)	1 µL
RNA Sample	2 µL

*The recommended volume of RNA sample is 2 µL, but a volume of 1 to 5 µL can also be used. Adjust the final volume of the reaction using nuclease-free water (4) to 20 µL.

2. For each qRT-PCR run, prepare a reaction containing nuclease-free water (4), as the no template control, and the HLVd positive control (5) instead of the RNA sample.
3. Run the experiment using the following program in the thermocycler:

Temperature	Time	
50°C	15 minutes	
95°C	30 seconds	
95°C	10 seconds	40 cycles
60°C	30 seconds	

4. Interpret data using the following table:

FAM (HLVd)	HEX (Cannabis Ctrl)	Results
$C_T < 35$	$C_T < 35$	Positive
$C_T > 35$	$C_T < 35$	Negative
$C_T > 35$	$C_T > 35$	Poor RNA quality or inhibited reaction