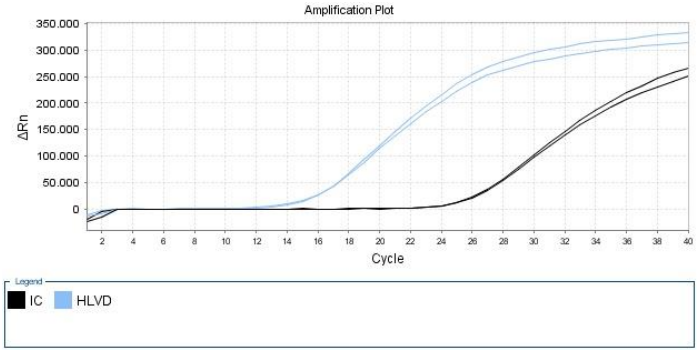
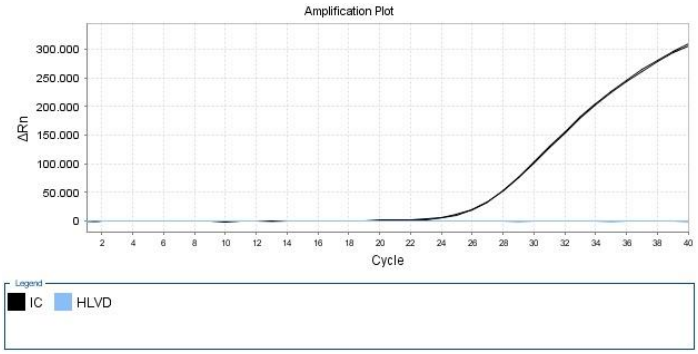
Positive Control –



Negative Control –



11. SPECIFICITY/INCLUSIVITY

Specificity is ensured by the selection of highly specific primers and probes. The primers and probes were checked for possible homologies to currently published sequences by sequence comparison analyses and demonstrated no or low cross-reactivity with other major relevant pathogens.

12. PERFORMANCE CHARACTERISTICS

Analytical sensitivity was determined by a 10-fold dilution series of a synthetic fragment of HLVd. The sensitivity is at least 3 copies/reaction for the target. The limit of detection (LoD95 = smallest number of copies of target which can be detected in 95% of cases) was determined by fifteen replicates at four different concentrations around the detection limit and is 9 target copies/reaction for HLVd.

BPMR – Production and Development, Lda. Amadora INOVA
Rua Henrique de Paiva Couceiro, 10
2700 – 453 Falagueira – Venda Nova Amadora, PORTUGAL
Tel: +351 211 451 410
E-mail: sales.support@biopremier.com
Website: http://www.biopremier.com

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BIOPREMIER

SUPREME
RT- PCR REAL TIME
DETECTION KIT
HLVD (Hop latent viroid)

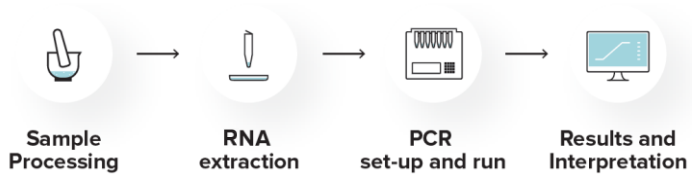
Ref: BIOPSFS-0064

1. PATHOGEN DESCRIPTION

Hop latent viroid (HLVd) is a single-stranded, circular infectious RNA that is dependent on its host plant's metabolism for replication. HLVd infection occurs worldwide in hops (*Humulus lupulus*) but it can also infect the cannabis plant. The HLVd-infected plants don't show obvious symptoms of infection but instead grow shorter with smaller leaves, which reduce the production of certain cultivars. HLVd is usually spread via infected tools or through cloning (cuttings taken from an infected mother). Because symptoms of HLVd are not always obvious in the vegetative stage, screening regularly by qPCR is the key to isolating the infected plants from their growing area.

2. INTENDED USE

BIOPREMIER SUPREME RT-PCR Real Time Detection Kit HLVd is designed for the detection of Hop Latent Viroid (HLVd) in a single PCR reaction based on one-step reverse transcription real-time PCR. The kit allows rapid and sensitive detection of RNA of HLVD in samples purified from plants.
The HLVD RNA detection is based on one-step reverse transcription real-time PCR (RT-PCR). A specific RNA sequence of the pathogen genome is transcribed into cDNA and amplified in a one-step PCR. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplificates.
BIOPREMIER SUPREME RT-PCR Real Time Detection Kit HLVd includes a PCR internal control (IC) to monitor for PCR inhibition in the sample, to validate the quality of the sample and detection results. The kit includes Master mix for the target and IC, primers and TaqMan® probes, labelled with non-fluorescent quenchers, as well as a positive control and a negative control. A probe-specific amplification curve at 530 nm (FAM channel) indicates the amplification of virus-specific RNA (HLVD). The IC is detected in Cy5 channel.
This test has been developed and validated for use with the Applied Biosystems® (ABI) 7500 instrument (Thermo Fisher Scientific) and LightCycler® 96 (Roche),but is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in **FAM** and **Cy5** channels.
This kit is designed for research use only not for use in diagnostic procedures.
The procedure includes the following main steps:



3. TRADEMARKS AND DISCLAIMER

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMR kit handbooks and user manuals can be requested from BPMR or your local distributor.

4. LIMITED LICENSE AGREEMENT

Use of this product signifies the agreement of the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMR grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

5. QUALITY CONTROL

In accordance with BPMR's ISO 9001, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

6. WARNINGS AND PRECAUTIONS

Molecular Biology procedures, such as RNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the samples. It is strongly recommended to have dedicated areas, materials and equipment for the RNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area.

- The user should always pay attention to the following:
- Read all the instructions provided before running the assay.
- Do not mix reagents from different batches.
- Wear proper PPE, including disposable gloves and laboratory coats.
- Store and extract positive material separately from all other reagents.

7. CONTENTS AND STORAGE CONDITIONS

The kit should be stored between -20°C and 5°C and protected from exposure to light. Repeated thawing and freezing (>5) should be avoided, as this may reduce the sensitivity. Considerate to freeze in aliquots to maintain the performance of the assay.
The kit contains reagents for 100 reactions / 500 reactions.

CONTENTS	UNITS	COMPOSITION
RNA reaction Mix (yellow cap)	1 tube (1 x 1060µl) 5 tubes (5 x 1060µl)	Reverse Transcriptase, RNase inhibitor, Taq Polymerase, Buffer and dNTPs
Assay Mix (Blue cap)	1 tube (1 x 735µl) 2 tubes (2 x 1838µl)	Primers and Probes
Negative Control (clear cap)	1 tube (1 x 105µl) 1 tube (1 x 525µl)	Nuclease-free water
Positive Control (red cap)	1 tube (1 x 105µl) 1 tube (1 x 525µl)	Target RNA

8. MATERIALS REQUIRED AND NOT SUPPLIED

- Microcentrifuge
- Laminar Air Flow Cabinets/PCR Cabinets
- Disposable powder-free gloves
- Micropipettes and nuclease-free filter tips
- Real time PCR instrument
- Tubes/Strips/Multiwell plates and accessories specific for each Instrument
- RNA extraction kit (example: BIOPEXT-0613)

9. PROCEDURE

9.1. SAMPLE PREPARATION

Depending on the specific characteristics of the sample, procedures like homogenization and grinding may be necessary before RNA extraction.

9.2. RNA EXTRACTION

Use a kit or protocol suitable for RNA extraction, such as BIOPREMIER RNA Viral Extraction Kit (ref: BIOPEXT-0613) or similiar. Follow the manufacturer's or author's instructions.
Best use RNA immediately after extraction and keep it on ice. Alternatively, use RNA stored at -20°C to -80°C and avoid prolonged exposure to room temperature, thaw on ice, and immediately refreeze the RNA.

9.3. PCR PREPARATION

A – PCR mix

Always wear gloves for all PCR procedures.

1. Thaw the kit solutions. Mix thoroughly (do not vortex the tube RNA reaction Mix - instead invert 2 to 3 times) and centrifuge briefly
2. Prepare the reactions, as described below:

CONTENTS	Nº OF SAMPLES	
qPCR reaction	**1**	**10 (10 + 1)**
RNA reaction Mix	10 µl	110 µl
Assay Mix	7 µl	77 µl
Total Volume	**17 µl**	**187 µl**

Note: Prepare the PCR reaction for each sample, or in alternative, prepare a Master Mix for the total number of reactions plus 10% (e.g. for 10 samples, prepare a volume for 11). In this case, prepare the Mix in a 1,5mL sterile, nuclease-free tube. Include 2 PCR reactions for the Positive and Negative controls.

3. Mix the prepared Mix by inverting the tube and centrifuge briefly
4. Dispense 17 µL aliquots of prepared Mix into the plate wells or PCR tube
5. For the negative control, pipette 3 µL of Negative Control tube (Clear Cap); Pipette 3 µL of RNA sample per well; and for the positive control, pipette 3 µL of Positive Control tube (Red Cap). Each PCR tube / well should have a final PCR volume of 20 µL
6. Centrifuge briefly the plate wells or PCR tubes
7. Place the reactions into the Real Time PCR instrument.

B – Program set up

Prepare the Real-Time PCR instrument according to the following temperature/time program:

PHASE	TEMPERATURE	CYCLES	TIME	ACQUISITION
Reverse Transcription	45 ºC	1	10 min	No
Polymerase Activation	95 ºC	1	2 min	No
Amplification	95 ºC	40	10 s	No
	60 ºC		1 min	Collect Data

Sample Volume: 20 µl
Passive reference dye: None
Detection format: 2 Color Hydrolysis Probe

TARGET	CHANNELS
Detection of HLVD	FAM | Excitation at 465 nm, Emission at 510 nm
Detection of Internal Control (IC)	Cy5 | Excitation at 618/610 nm,Emission at 660/670 nm

10. DATA ANALYSIS

For analysis of PCR results, select fluorescence display options. Samples with positive Ct-values are considered positive.
Important: Please, also check amplification curves, not only Ct values. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control. Adjust the Threshold, if necessary. Sample results should be assessed after the positive and negative controls have been examined and determined to be valid. If the results of controls are not valid, the sample results cannot be interpreted.
Interpretation of PCR-data:

a) Controls

To validate the assay, the controls must have the following results:

	HLVD DETECTION FAM	IC DETECTION Cy5
Negative Control	Negative	Positive
Positive Control	Positive	Positive

Note that if the controls do not match these results, the experiment must be repeated.

b) Samples

Interpretation of sample results is summarized in the following table:

HLVD DETECTION FAM	IC DETECTION Cy5	INTERPRETATION
Positive	Positive/Negative**	Sample Positive for HLVD
Negative	Positive	Sample Negative for HLVD
Negative	Negative	Invalid Result*

*When both HLVD and IC detection are Negative, this means the presence of PCR inhibitors in the sample. Dilute the sample or perform another RNA extraction.
** High RNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.

Note: If the sample is weakly positive (approx. Cq > 35) in FAM channel, there is a low RNA concentration. In this case the results should be interpreted carefully. Extract again and concentrate the RNA from the sample. Repeat the PCR.