

*One-step real-time TaqMan Probe RT-PCR for Hop Latent Viroid Detection from plant samples*

Catalog	Kit Size (RXN)
<b>TBS42015-50</b>	50
<b>TBS42015-100</b>	100
<b>TBS42015-200</b>	200

## DESCRIPTION

TaqMan RT-PCR Hop Latent Viroid (HLVd) Detection Kit is one step real-time reverse transcription polymerase chain reaction (RT-PCR) assay intended for the detection of HLVd infection in plant samples. It combines both reverse transcription (RT) and Taqman probe-based PCR amplification to occur in a single reaction tube. The kit is optimized for the two reactions in a real-time “single step”. This One-step qRT-PCR assay offers the end-users an efficient, easy to use, and reliable alternative to conventional “two-step” sequential qRT-PCR for detecting HLVd.

TaqMan RT-PCR Hop Latent Viroid Detection Kit contains all components for RT-PCR reaction including RT-PCR enzymes, primer-probe, internal control, positive control, negative control, and buffer. The HLVd target gene is labeled with fluorescence Fam, and internal control is labeled with Hex.

## APPLICATIONS

This kit is used for detection of HLVd infection in plant samples.

## KEY FEATURES

- One-step complete qRT-PCR in a single tube.
- Reduce contamination in operating process.
- Accurate detection and quantification of HLVd target gene.
- 4x RT-PCR mix makes easier to adjust the sample size in one-tube reaction.

## KIT CONTENTS

Component	50RXN	100RXN	200RXN
4X RT-PCR Master Mix	0.25mL	0.5 mL	1mL
10X HLVd Primer-probe	0.1mL	0.2 mL	0.4mL
HLVd Positive Control	25 µL	50 µL	100 µL
HLVd Negative Control	25 µL	50 µL	100 µL
4x RT-PCR Buffer	0.25 mL	0.2 mL	0.4 mL
DEPC Water	1.5 mL	1.5 mL	2x1.5mL

## STORAGE CONDITIONS

Store all components at -20°C in a non-frost-free freezer. Shelf life is 12 months after recipient. The kit is shipped on ice.

## PROTOCOL

**Precautions:** RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR and subsequent reaction analysis should be performed in separate areas. The use of “clean”, automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended.

1. RNA isolation is performed with a suitable approach. We recommend our Hybrid-R RNA purification kit (catalog: 305-101) for RNA extraction from plant samples.

2. Prepare the following reaction mixture on ice

Components	Reaction Vol.:20 µl	Concentration
Total RNA	Variable	5 pg - 1 µg/rxn
RT-PCR Mix (4x)	5 µl	1X
Primer-probe Mix (10x)	2 µl	1x
RT-PCR Buffer (4x)	5 µl	1x
DEPC water	Adjust to the final volume to 20	

**Positive or Negative Control: 2µl/each well.**

The HLVd target gene is labeled with **Fam**, and internal control is labeled with **Hex**

### Note:

1. Gene specific primers and probe must be used for RT-qPCR amplification.
2. Please ensure no salt crystals are present in the RT-qPCR Mix before use. If salt crystals are observed, mix until crystals are completely dissolved and absent.
3. For Positive and negative control, use 2 µl of Positive or negative control to replace the RNA sample.
3. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
4. Program the thermal cycler so that cDNA synthesis is followed immediately by qPCR amplification.

Step	Temperature	Duration	Cycle(s)
1	50°C	20 mins	1
2	95°C	1 min	1
3	95°C	15 secs	40
	60°C	60 secs	

## Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to qRT-PCR reaction.

## DATA ANALYSIS

After completion of the run, save and analyze the data following the instrument instructions. Analyses should be performed separately for each fluorescence channel using a manual threshold setting. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal. The procedure chosen for setting the threshold should be used consistently.

The results can be interpreted as below:

**Positive:** HLVd gene Ct value is in 12-36, and internal control (Hex), Positive control, and Negative control are normal.

**Negative:** HLVd gene Ct value is  $\geq 37$ , and internal control (Hex), Positive control, and Negative control are normal.

### RELATED PRODUCTS

TBS6025: Microbial DNA Magnetic Extraction

TBS42018: Trichothecene-producing Fusarium Species

TaqProbe qPCR Detection

TBS42019: Fusarium Species qPCR Detection

TBS 42020: Universal Aspergillus qPCR

TBS42021: Aspergillus Flavus qPCR

TBS42022: Aspergillus Fumigatus qPCR

TBS42023: Aspergillus Niger qPCR

TBS42024: Aspergillus Terreus qPCR

TBS42025: 4-In-1 Aspergillus qPCR

TBS42026: O157H7 E. Coli qPCR

TBS42027: STEC qPCR

TBS42028: Salmonella qPCR

TBS42029: STEC and Salmonella Multiple qPCR

TBS42030: Mycoplasma Detection qPCR

TBS42031: Listeria Monocytogenes qPCR

TBS42032: Listeria Genus qPCR

TBS42033: Bacillus Cereus qPCR

TBS42043: Bacillus Species qPCR

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