Cryptic virus (CV)-Hop Latent Viroid (HLVD)-Lettuce Chlorosis (LCV) Detection from Hem Plant

Catalog	Kit Size (RXN)
TBS42016-50	50
TBS42016-100	100
TBS42016-200	200

DESCRIPTION

CV-HLVD-LCV Multiplex RT-PCR Detection Kit is used for the detection of cryptic virus (CV), hop latent viroid (HLVD), and lettuce chlorosis (LCV) from cannabis plant in one step RT-PCR reaction. 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 CV, HLVd, and LCV from plant samples.

Tribioscience Multiplex RT-PCR Detection Kit contains all components for RT-PCR reaction including RT-PCR enzymes, primer probes, internal control, positive control, negative control, and buffer. The 3 target genes are labeled with a specific fluorescence color: Cy5 for Cryptic virus, Fam for HLVD, ROX for lettuce chlorosis virus, and Hex for internal control.

APPLICATIONS

This kit is used for the detection of CV, HLVd, and LCV infection in plant samples.

KEY FEATURES

- One-step complete qRT-PCR in a single tube.
- Reduce contamination in the operating process.
- Accurate detection and quantification of HLVd target gene.
- 4x RT-PCR mix makes it 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 Primer-probe Mix	0.1mL	0.2 mL	0.4mL
Positive Control	50 µL	100 µL	200 µL
Negative Control	50 µL	100 µL	200 µL
4x RT-PCR Buffer	0.25 mL	0.5 mL	1.0 mL
DEPC Water	1.0 mL	1.0 mL	2x1.0mL

STORAGE CONDITIONS

Store all components at -20°C in a non-frost-free freezer.
Shelf life is 12 months after receipt. 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. *Note: Nuclease Removal (TBS6013) is useful for removing RNase contamination in the working area and tools.*

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 to replace RNA sample: 4µl/well.
CV is labeled with Cy5, HLVd is labeled with Fam, LCV is labeled with ROX, and internal control is labeled with Hex

Note:

1. Gene specific primer-probe mix 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. 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

The results can be interpreted as below:

Positive: Target gene: CV, or HLVD or LCV Ct value is in 12-36, and internal control (Hex), Positive control, and Negative control are normal.

Negative: Target gene, CV, or HLVd, or LCV Ct value is ≥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
TBS42015: HLVD RT-qPCR detection
TBS42019: Fusarium Species qPCR Detection
TBS42020: 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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365 San Aleso Ave, Sunnyvale, CA 94085, USA Website: www.tribioscience.com
Tel: 408-498-0197, Fax: 650-618-5498 Email: order@tribioscience.com