

Candidatus Liberibacter TaqMan PCR Kit
Product# TM64300, TM64350

Product Insert

Intended Use

Norgen's *Candidatus Liberibacter* TaqMan PCR Kit is designed for the detection of *Candidatus Liberibacter* specific DNA in a real-time PCR based on the use of TaqMan® technology. This kit is designed for research use only and not for use in diagnostic procedures.

Background Information

Citrus Huanglongbing (HLB), previously known as citrus greening, constitutes the most economically devastating disease of citrus worldwide, being present in around 40 countries. HLB causes severe symptoms, depending on the age and the number of infections per plant. The disease is caused by the fastidious gram-negative α -proteobacteria *Candidatus Liberibacter asiaticus* (Las), *Ca. L. africanus*, and *Ca. L. americanus* as well as some phytoplasmas. The bacterium propagates within the phloem of citrus plants producing die-back, yellow shoots, blotchy mottles on leaves, and off-tasting and malformed fruit. The 'Ca. Liberibacter' bacterium is transmitted by the psyllids *Trioza erytreae* in Africa and *Diaphorina citri* in Asia and the Americas. A variety of diagnostic methods have been developed to supplement or confirm field-based diagnosis of HLB however, end-point PCR-based and real-time quantitative methods are currently the most widely used methods in plant and insect tissues to detect and identify HLB-Liberibacters.

Product Description

Norgen's *Candidatus Liberibacter* TaqMan PCR Kit is designed for the detection of *Candidatus Liberibacter* specific DNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures. The detection of *Candidatus Liberibacter* specific DNA is based on TaqMan PCR providing a simple, reliable and rapid result for the detection of *Candidatus Liberibacter* infection. Norgen's *Candidatus Liberibacter* TaqMan PCR Kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The *Candidatus Liberibacter* TaqMan PCR Kit comprises Master Mix for the target and PCR control detection, Primer & Probe Mix, as well as a positive control and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.

Norgen's *Candidatus Liberibacter* TaqMan PCR Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad CFX96 Touch™ Real-Time PCR Detection System

Kit Components

Component	Product # TM64300 (24 preps)	Product # TM64350 (100 preps)
MDx TaqMan 2X PCR Master Mix	350 μ L	3 x 350 μ L
Candidatus Liberibacter Primer & Probe Mix	70 μ L	3 x 70 μ L
Candidatus Liberibacter Positive Control	50 μ L	3 x 50 μ L
Nuclease-Free Water (Negative control)	1.25 mL	1.25 mL
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Storage Conditions and Product Stability

- All kit components should be stored at -20°C upon arrival
- Repeated thawing and freezing (> 2 x) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.
- All reagents can be stored for 1 year at -20°C without showing any reduction in performance.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument with FAM and HEX filter channel
- DNA Purification Kit
 - The kit is compatible with all DNA purification kits that yield high quality, inhibitor-free DNA
 - **Recommended Purification Kit:** Norgen's Plant/Fungi DNA Isolation Kit (Cat. 26200, 26900)
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- PCR reaction preparation station (Optional)

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *Candidatus Liberibacter* TaqMan PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Norgen's *Candidatus Liberibacter* TaqMan PCR Kit is intended for research purposes only. It is not intended for diagnostic use.
- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Dispose of unused kit reagents and specimens according to local, provincial or federal regulations.

- Do not substitute or mix reagents from different kit lots or from other manufacturers. Do not use components of the kit that have been stored for more than 1 year.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the *Candidatus Liberibacter* genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.
- Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

Instructions for Use

A. Sample Preparation

Purified DNA is the starting material for Norgen's *Candidatus Liberibacter* TaqMan PCR Kit. The quality of the DNA template will have a major impact on the performance of the *Candidatus Liberibacter* detection test. The user must ensure that the method used for DNA purification is compatible with TaqMan PCR. We recommend the use of **Norgen's Plant/Fungi DNA Isolation Kit (Cat. 26200, 26900)**. Norgen's Plant/Fungi DNA Isolation Kit has been fully validated with Norgen's *Candidatus Liberibacter* TaqMan PCR Kit.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 3 minutes at 20,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the DNA. This will help to prevent the carry-over of any ethanol into the purified DNA, as ethanol is known to be a strong inhibitor of PCR. **Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the DNA.**

B. TaqMan PCR Assay Preparation

Notes:

- Before use, suitable amounts of all TaqMan PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- For every TaqMan PCR run, one reaction containing *Candidatus Liberibacter* Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per TaqMan PCR run is 6.
- To avoid any contamination while preparing the TaqMan PCR assay, follow the order outlined in Tables 1, 2 and 3 below to prepare the Negative Control, Detection Assay and Positive Control:
 1. Prepare the PCR Negative Control (Table 1)
 2. Prepare the PCR *Candidatus Liberibacter* Assay (Table 2)
 3. Prepare the PCR Positive Control (Table 3)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) Primer & Probe Mix; 3) Primer Set; and 4) the Sample DNA or Positive Control).

1. For each TaqMan PCR set, prepare one no template control PCR as shown in Table 1 below:

Table 1. TaqMan PCR Negative Control Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix)
Nuclease-Free Water	8 µL
MDx TaqMan 2X PCR Master Mix	10 µL
Candidatus Liberibacter Primer & Probe Mix	2 µL
Total Volume	20 µL

2. Prepare the PCR reaction for sample detection as shown in Table 2 below.

Table 2. TaqMan PCR *Candidatus Liberibacter* Assay Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix)
Nuclease-Free Water	5 µL
MDx TaqMan 2X PCR Master Mix	10 µL
Candidatus Liberibacter Primer & Probe Mix	2 µL
Sample DNA*	3 µL
Total Volume	20 µL

* The recommended amount of sample DNA to be used is 3 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

3. For each PCR set, prepare **one** positive control PCR as shown in Table 3 below:

Table 3. TaqMan PCR Positive Control Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix)
MDx TaqMan 2X PCR Master Mix	10 µL
Candidatus Liberibacter Primer & Probe Mix	2 µL
Candidatus Liberibacter Positive Control (PosC)	8 µL
Total Volume	20 µL

4. **OPTIONAL** - Experienced User Protocol for PCR Reaction Preparation for Multiple Samples

Follow Table 4 and the instructions below to prepare a PCR reaction mix for multiple samples. The below example is to set up 10 PCR reactions.

Table 4. Set-Up of PCR Reaction Mix for 10 PCR Reactions

Components	Volume per 10 + 1 Reactions
MDx TaqMan 2X PCR Master Mix	110 μ L
Candidatus Liberibacter Primer & Probe Mix	22 μ L
Total Volume	132 μ L

1. Aliquot 12 μ L of the PCR reaction mix to PCR tubes or wells on a 96-well PCR plate.
2. For the negative control, add 8 μ L of Nuclease free water and close the PCR tube cap.
3. For the detection samples, add 5 μ L of water and 3 μ L of template (DNA) to samples. Close the PCR tube cap.
4. For the positive control, add 8 μ L of *Candidatus Liberibacter* Positive Control (PosC) and close the PCR tube cap.
5. Spin the tubes or 96-well plate briefly.
6. The final PCR reaction volume is 20 μ L.

C. *Candidatus Liberibacter* TaqMan PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run TaqMan PCR assay.

Table 4. *Candidatus Liberibacter* TaqMan PCR Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1	95°C	3 min
Cycle 2 (40x)	Step 1	95°C	15 sec
	Step 2	60°C	30 sec

D. *Candidatus Liberibacter* TaqMan PCR Assay Interpretation

Table 5. Interpretation of Assay Results

FAM (Target detection)	HEX (PCR validation)	Result
+	+	Positive
-	+	Negative
-	-	PCR inhibited

For results obtained that are not covered in Table 5, please refer to the Frequently Asked Questions.

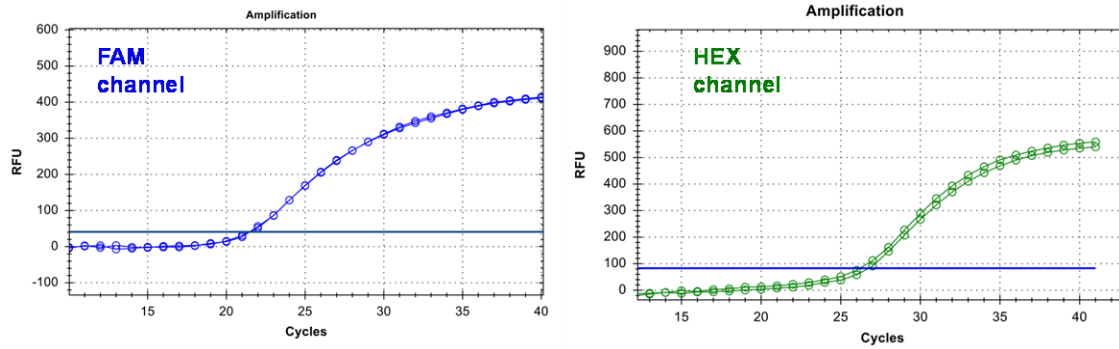


Figure 1. Example of TaqMan PCR Positive result. Both PCR signals above the baseline from FAM and HEX channel indicate the successful PCR.

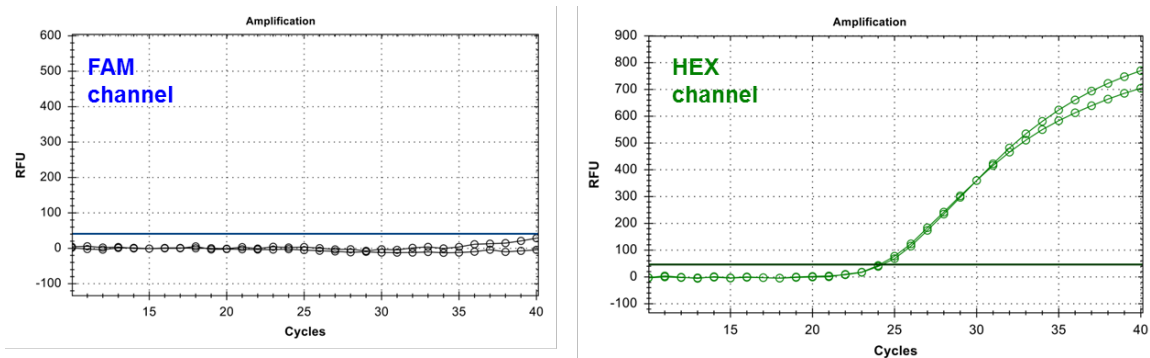


Figure 2. Example of TaqMan PCR Negative result. No target DNA was detected in FAM channel but amplification signal from HEX indicates the successful PCR.

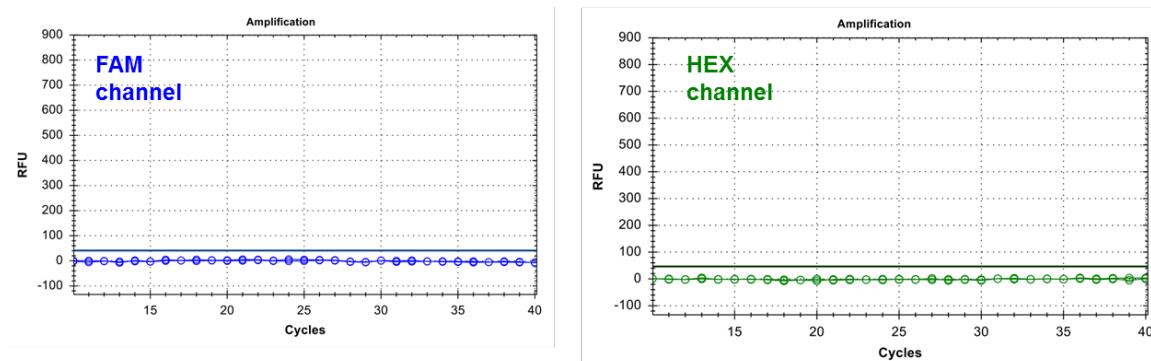


Figure 3. Example of TaqMan PCR inhibition result. No signal from both FAM and HEX channel was detected. It is suggested to repeat the sample preparation using recommended kit for DNA purification.

E. *Candidatus Liberibacter* TaqMan PCR Assay Specificity

The specificity of Norgen's *Candidatus Liberibacter* PCR Detection Kit is first and foremost ensured by the selection of the *Candidatus Liberibacter* -specific primers, as well as the selection of stringent reaction conditions. The *Candidatus Liberibacter* primers were checked for possible homologies to all microorganisms in GenBank published sequences by sequence comparison analysis.

Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's *Candidatus Liberibacter* TaqMan PCR Detection Kit is designed to test 24 or 100 samples. For every 8 samples, a non-template control (Nuclease-Free Water) and a Positive Control must be included. It is preferable to collect and test 8 samples at a time.

2. **How should it be interpreted if no PCR control signal (HEX) is detected while the target specific signal (FAM) is detected in the positive control?**
 - Tested samples(s) can be considered positive. It could happen when too much target DNA template was added due to the preferential amplification on the target.
3. **How should it be interpreted if the target specific signal (FAM) and the PCR control signal (HEX) are detected in the negative control?**
 - It could happen when there is carryover contamination and PCR inhibition. Repeat the assay using fresh aliquots and clean pipette tips.
4. **How should it be interpreted if no target signal (FAM) is detected in positive control?**
 - It could happen when the positive control was not added. Repeat the assay.

Related Products	Product #
<i>Candidatus Liberibacter</i> TaqMan Probe/Primer and Control Set	TM64310
Plant/Fungi DNA Isolation Kit	26200, 26900

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

Norgen's *Candidatus Liberibacter* TaqMan PCR Kit is designed for the detection of *Candidatus Liberibacter* specific DNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen's *Candidatus Liberibacter* TaqMan PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR.

Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

The presence of PCR inhibitors may cause false negative or invalid results.

Potential mutations within the target regions of the *Candidatus Liberibacter* genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

The respective user is liable for any and all damages resulting from application of Norgen's *Candidatus Liberibacter* TaqMan PCR Kit for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the

laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

TaqMan is a registered trademark of Roche Molecular Systems, Inc

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