DNAble® Detection Kit for HLB Pathogen in Citrus Petiole

Catalog DF-028-PT *Part # 11783*

Highlights:

- *Molecular Detection of* Liberibacter asiaticus *DNA in citrus plant petiole samples*
- *Rapid amplification and detection in 15 minute assay*

Contents of DNAble Kit:

- *RB1 Reaction Buffer*
- *HLB Master Mix*
- *Flat caps*
- *MB5 Extraction Buffer in dropper bottle*
- *D1 Buffer in dropper bottle*
- *1.5 mL extraction tubes*

Materials Not Provided:

- *MiniPet: 10 µL and 50 µL**
- *Pipette Tips (200 µL)*
- *Marker*
- *Timer*
- *3 mm Harris punch**
- *AmpliFire DNAble Reader**
- *Dry heat block capable of 95°C with insert suitable for 2 mL tubes**

**Available through EnviroLogix*

Intended Use

This test kit is intended for qualitative detection of DNA from *Liberibacter asiaticus,* the bacteria that causes huanglongbing (HLB), also known as citrus greening disease. The results of this test may facilitate rapid, point of need detection of HLB in citrus leaf petioles.

How the Test Works

DNAble is an isothermal nucleic acid amplification technology enabling rapid amplification of a specific DNA target. In this test, samples are collected, processed, and added to the reaction buffer. The reaction buffer containing sample is then transferred to the lyophilized master mix, containing all the reagents needed to specifically recognize, amplify and detect the *Liberibacter asiaticus (L. as)* specific DNA.

The amplified *L. as* DNA is detected in real-time and the results are displayed and interpreted within 15 minutes using the AmpliFire Reader, powered by DNAble.

Precautions and Notes

DNAble is a highly sensitive assay. Therefore the following precautions are recommended to reduce the chance of sample contamination:

- clean the work stations and pipettes before and after use with 10% bleach solution
- it is recommended to physically separate sample preparation activities from DNAble assay activity
- do not reuse kit disposables
- use fresh pipette tips for each sample
- discard used tips in a sealed container containing 10% bleach solution
- use careful pipetting techniques to avoid cross-contamination between samples; avoid reaching over or pipetting over open tubes
- wear gloves and change between handling of samples

Important: Never open reaction tubes after reaction has occurred, as this will release amplified material into the environment and may contaminate subsequent reactions. Care should be taken when disposing of run reaction tubes to avoid possibility of tube leakage. Place completed reaction tubes back in original zippered pouch prior to disposal.

Kit Components

- RB1 Reaction Buffer: Provided in green 8-well strip tubes (6 strips)
- HLB Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (6 strips)
- Flat Caps: used for capping the clear tubes prior to assay start (6 strips)

- MB5 Extraction Buffer: 30 mL of extraction buffer in dropper bottle
- D1 Buffer: 30 mL of D1 Buffer in dropper bottle
- 2.0 mL locking lid extraction tubes: One bag of 20 tubes for sample extraction

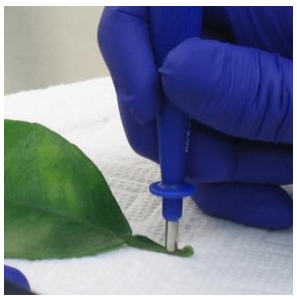
Before Testing

- Remove needed DNAble Kit reagents from refrigerated storage. Allow reagents to come to room temperature before opening sealed white pouches.
- Turn on the 8-well AmpliFire Reader. Allow AmpliFire to warm to 56ºC.
- Turn on heat block to 95°C. Allow warming to temperature for at least 30 minutes.
- Ensure that all assay reagents, extracted sample, pipettes and flat caps are ready for use.

Petiole Sample Collection and Preparation

1. **Collect sweet orange leaf and petiole sample.** If sample will not be tested immediately, store refrigerated for 1-2 days, or frozen at -20°C for longer term storage.
2. Using a dropper bottle, add **500 µL of MB5** Extraction Buffer to the tube (to the 0.5 mL line).
3. Using a clean, dry Harris punch, punch out **four pieces of petiole** starting from the bottom of the petiole and collecting along the center. (Collect as little leafy tissue as possible.)
4. Add these 4 petiole punches to the buffer in tube.
5. Heat sample in pre-warmed heat block at **95°C for 5 minutes**.
6. After 5 minutes, remove sample tube from heat and **add D1 Buffer to the tube up to the 1.0 line** (500 µL of D1 Buffer added to tube).
7. **Invert sample tube 10 times** to mix.
8. 10 µL of each sample will be used for the assay in the next section.


a. Place 500 µL of MB5 in tube


b. Use 3 mm Harris punch to sample 4 petiole punches


c. Eject punches into extraction tube


d. Heat tubes in pre-warmed dry block at 95°C for 5 minutes


e. Add 500 µL of D1 Buffer to heated extract; invert 10X to mix

How to Run the DNAble Assay

DNAble assay protocol

1. On the AmpliFire screen, select "**Execute Reaction**" then "**Scan Product Code**". Use the barcode on the master mix foil pouch to scan the HLB protocol on the AmpliFire Reader. HLB_Lot # will display. Select "**Next**".
2. Under "Reaction Name" enter the **run information.** Select "Next" and then "Finish" to skip well-specific sample entry.

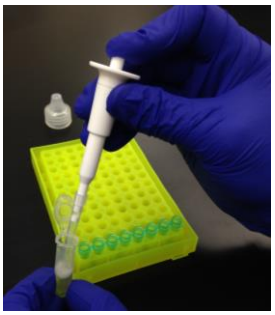
3. Remove clear Master Mix tubes from the foil pouch and gently tap down to ensure that the white pellet is at the bottom of the tubes.

   Important: *Label tube for orientation at the top of the tube (writing on the bottom half of the tube will interfere with results interpretation).*

4. Remove green RB1 Reaction Buffer tubes from bag and tap down to ensure that the liquid is at the bottom of the tube. Label the tubes.

5. Using a 10 µL white MiniPet, **transfer 10 µL of petiole extract** (from Step 8 of Petiole Sample Preparation Section) to the green reaction buffer strip. Repeat for samples 2-8.

6. Recap with green strip caps and gently flick tubes to mix sample with buffer. Tap down to ensure that all liquid is at the bottom of the tube.



Transfer 10 µL of petiole extract to reaction buffer strip

Transfer 50 µL of petiole mixture to Master Mix strip

7. Remove clear Master Mix tubes from the foil pouch and gently tap down to ensure that the white pellet is at the bottom of the tubes.

   Important: *Label tube for orientation at the top of the tube (writing on the bottom half of the tube will interfere with results interpretation).*

8. Using a 50 µL yellow MiniPet, **transfer 50 µL** from the green reaction buffer strip to the master mix tube. Repeat for samples 2-8.

   Important: *Simply dispense buffer during pipetting step; do not mix up and down.*

9. Recap with provided "Flat Caps".

   Important: *Ensure that the tubes are* ***completely sealed*** *with flat caps.*

10. Gently tap down on the resuspended, capped master mix two times. Inspect to ensure that no air bubbles are present within the sample volume (a bubble at the top is fine).

11. When the instrument is ready push "**Start**". Place resuspended, capped clear strip tube into the AmpliFire DNAble Reader and press "**Ok**".

12. After 15 minutes, the AmpliFire DNAble Reader will produce a short beeping sound and display final results. Results will be interpreted as Not Detected (-), Positive (+), or Invalid (!).

    Positive results may be interpreted prior to assay completion, but the full assay time must be complete for interpretation of negative results.

13. After completion of the assay, carefully **remove run reaction strip tubes from instrument and place in opened foil pouch** (used to store master mix), seal and discard in waste container.

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