# DNAble® Detection Kit for HLB Pathogen in Psyllids

*Catalog DF-028-PS* *Part # 11764*

## Highlights:

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

## Contents of DNAble Kit:

- *HLB Master Mix*
- *Flat caps*
- *MB1 Extraction Buffer in 15 mL dropper bottle*
- *1.5 mL extraction tubes*
- *Blue Pestles*

## Materials Not Provided:

- *Minipets: 50 µL**
- *Pipette Tips (200 µL)*
- *Marker*
- *Timer*
- *AmpliFire DNAble Reader*

**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 Asian citrus psyllids, the carrier of this pathogen.

## 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 lyophilized master mix. The resuspended master mix contains 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

- HLB Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (3 strips)
- Flat Caps: used for capping the clear tubes prior to assay start (3 strips)

- MB1 Extraction Buffer: 15 mL of extraction buffer in dropper bottle
- 1.5 mL Extraction Tubes: Two bags of 25 tubes for sample extraction
- Blue Pestles: One bag of 50 pestles for sample maceration

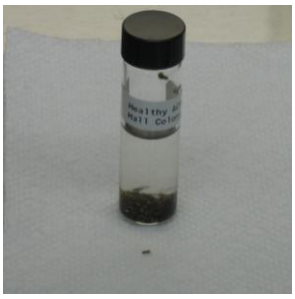
## Before Testing

- 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 DNAble Reader. Allow AmpliFire to warm to 56ºC.
- Ensure that all assay reagents, extracted sample, pipettes and flat caps are ready for use.

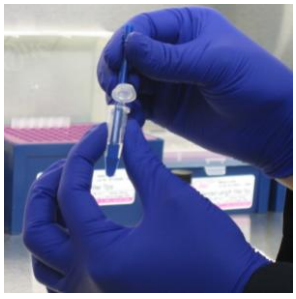

## Psyllid Sample Collection and Preparation

1. Wear gloves and change between samples.
2. Collect psyllids from the field and place into 90% ethanol solution. Keep on ice or frozen at -20°C until testing.
3. Using the dropper bottle, carefully add 250 µL of MB1 Extraction Buffer. Add to the 0.25 mL line (in between the labeled 0.1 mL and 0.5 mL lines).
4. At the time of testing, place each psyllid on a clean paper towel for 2 minutes to briefly dry off the ethanol. Clean forceps or a pipette tip with the end cut off work well for psyllid collection from ethanol.
5. Place dried psyllid(s) in a sample extraction tube containing buffer. **One to 10 psyllids may be pooled together into a single sample tube**.
6. **Macerate psyllids in buffer with a blue pestle.** Ensure that each psyllid has been macerated.
7. Gently invert the tube 5 times to mix, then allow sample to settle for at least 3 minutes.
8. **50 µL** of each sample will be used for the assay in the next section.

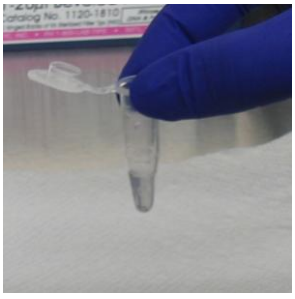
a. Place 250 µL of MB1 in tube



b. Dry psyllids for several minutes, then add to tube with MB1



c. Macerate psyllid(s) using pestle



d. Allow to settle for several minutes; 50 µL will be used in the assay

## How to Run the DNAble Assay

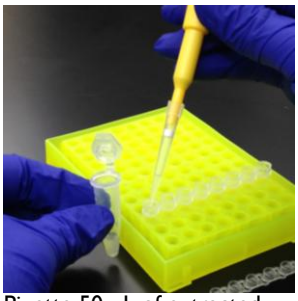
### DNAble assay protocol

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.

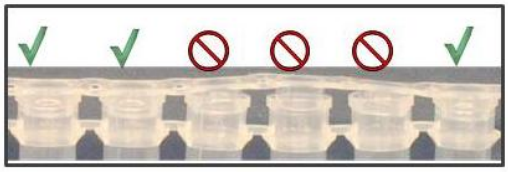
   ***Important:*** *Label tube for orientation at the top of the tube (writing on the bottom half of the tube will interfere with results interpretation).*


Pipette 50 µL of extracted sample to Master Mix

4. Using a 50 µL yellow MiniPet, **add 50 µL of clear solution from extracted psyllid sample** (from Step 8 of Psyllid Sample Preparation Section) directly to the lyophilized master mix. Avoid pipetting any particulates from macerated insect. Repeat for samples 2-8.
   a. Simply dispense the sample into the lyophilized master mix without pipetting up and down.

5. Recap with provided "Flat Caps".

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



6. 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).

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

8. 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.

9. 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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