DNAble® Molecular Detection Kit for MON 88302 Canola Leaf and Bulk Seed

Catalog No. DF-017 *Part # 12771*

Highlights:

- *Molecular Detection of MON 88302 Event in canola leaf and bulk seed.*
- *Rapid amplification and detection in a 10 minute assay*

Contents of DNAble Kit:

- *Reaction Buffer*
- *MON 88302 Event Master Mix*
- *Flat Caps*

Materials Not Provided:

- *Pipette capable of delivering 25 µL*
- *Multichannel pipette capable of delivering 50 µL*
- *Filter Pipette Tips*
- *Marker*
- *Bleach*
- *Sample Extraction Set 5**
- *AmpliFire DNAble Reader**

**Available through EnviroLogix*

Intended Use

This test kit is intended for rapid qualitative detection of DNA of MON 88302 Event as present in canola leaf and seed.

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 the sample extract is added to the reaction buffer. The reaction buffer containing extract is then transferred to the lyophilized Master Mix, containing all the reagents needed to specifically recognize, amplify and detect the MON 88302 Event-specific DNA in canola leaf or bulk grain samples.

The amplified MON 88302 Event-specific DNA is detected and the results are displayed and interpreted at 10 minutes using our 8-well AmpliFire DNAble Reader.

Intended User

DNAble is designed to be simple and user friendly. It is designed for use by personnel with appropriate training in Molecular Assay techniques. Training specific to the DNAble assay will be provided by EnviroLogix; contact Technical Service or visit envirologix.com for more information.

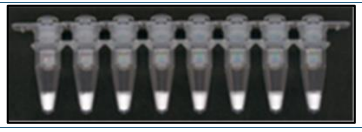
Precautions and Notes

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, including replicates from the same sample extract
- 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 disposable gloves when handling 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. If contamination is suspected, follow cleaning instructions (www.envirologix.com/support/dnable/) or contact Technical Support.

Kit Components

• Reaction Buffer: Provided in green 8-well strip tubes (6)	
• MON 88302 Event Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (6 strips).	

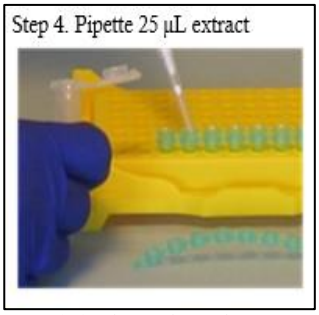
- Flat Caps: used for capping the clear tubes prior to assay start (6 strips)

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 using power button on the right side of the instrument.
- Ensure that all assay reagents, extracted sample, pipettes and flat caps are ready for use.

Sample Preparation

1. Follow **Sample Extraction Set 5** product insert for sample preparation, extraction, and thermal denaturation.
2. Seed samples are run in duplicate and interpreted as a result set. Leaf samples are run as a single replicate.
3. Remove green Reaction Buffer strip tubes from the kit. Mark the left end tube to note orientation.
   IMPORTANT: *Tap down or centrifuge green strip tube to ensure that the entire buffer volume is at the bottom of the tubes prior to opening.*
4. Pipette **25 µL of prepared sample extract 1** (from Step 1) into one well of the green Reaction Buffer 8-well strip.



   a. If testing <u>seed</u>, pipette **another 25 µL from the same extract 1** into the second well, and subsequent samples in duplicate, using a fresh pipette tip for each.

      Seed: Double replicates S1 S1 S2 S2 S3 S3 S4 S4

   b. If testing <u>leaf</u>, pipette **25 µL of sample extract 2** into the second well, and subsequent samples singly, using a fresh pipette tip for each.

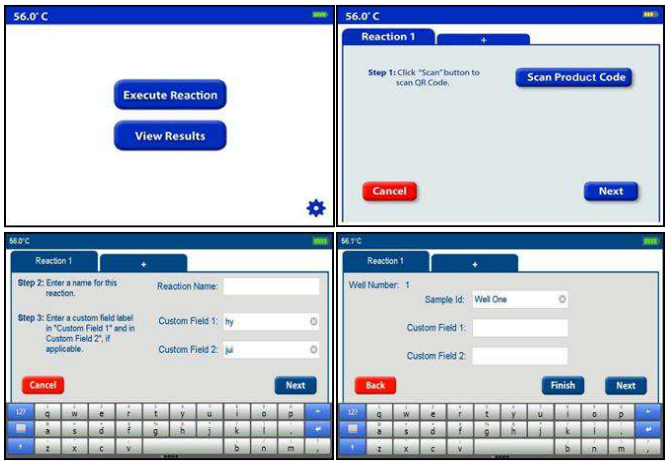
      Leaf Tissue: Single replicates S1 S2 S3 S4 S5 S6 S7 S8

   <u>IMPORTANT - FOR LEAF SAMPLES: Tube should be left undisturbed in rack prior to and during pipetting.</u>
   Carefully aspirate **25 µL** from the top of the extract, taking care not to disturb the buffer particles that have settled at the bottom of the tube.

5. Recap Reaction Buffer strip tubes and tap down to ensure all liquid is at the bottom of the tube.

How to Run the DNAble Assay

DNAble assay protocol



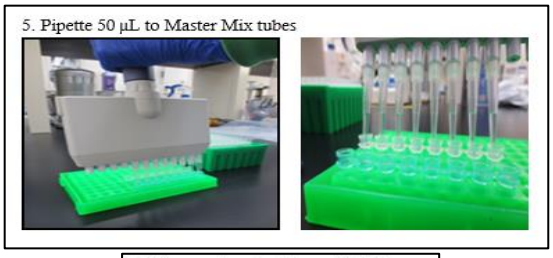
1. On the AmpliFire screen, select "**Execute Reaction**" then "**Scan Product Code**". Use the barcode on the Master Mix foil pouch to scan the MON 88302 Event protocol on the 8-well AmpliFire Reader. **"DF-017"** will display. Select "**Next**".

2. Under "Reaction Name" enter an appropriate reaction description**. This description is placed at the beginning of the file name.** Select "Next"

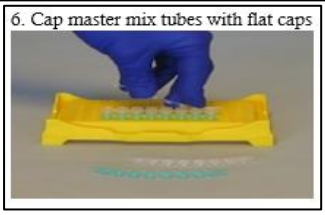
3. To enter specific sample, field or other information for Wells 1 through 8, input information in the Sample ID field and click "Next" to advance to each Well. Select "Finish" to skip well-specific sample entry.

4. 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: *Mark Flat Cap for orientation of the clear Master Mix tubes (writing on clear tubes will interfere with results interpretation or leave marker residue in instrument).*

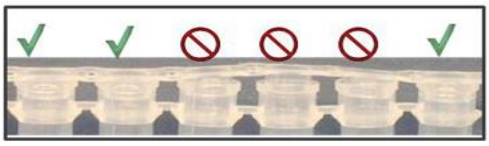


5. Using a multichannel pipette set to 50 µL, transfer the entire **50 µL** of liquid from green Reaction Buffer tubes to clear Master Mix tubes. If buffer in green tube was not entirely at the bottom, less than 50 µL could be transferred; this should not affect results. Discard clear domed caps from green Buffer Tubes and Master Mix Tubes. Do **not** mix within the clear tube.



6. **Cap** Master Mix tubes with provided **Flat Caps** strip and mark the left end tube to note orientation.

   Important: *Ensure that the tubes are **completely sealed** with Flat Caps as indicated by green check marks in the image (right).*



7. Gently flick down on the resuspended, capped Master Mix to ensure that no bubbles are at the bottom of the tube and that Master Mix is fully resuspended.

8. Inspect tube to ensure that **no air bubbles are present within the sample volume** (a bubble at the top is fine) and that **cap is completely sealed.**

9. When the strip is ready select "**Start**". Place resuspended, capped clear strip tube in instrument and press "**Ok**".
10. After **10 minutes**, the AmpliFire will produce a short beeping sound and display final results. Results will be interpreted as Not Detected (-), Positive (+) or Invalid (!)
    - The full assay time must be complete for result interpretation. (Empty wells will be interpreted as Not Detected.)
    - **Invalid** denotes that the internal control did not amplify as expected and indicates that either the sample has inhibited the reaction or that something was wrong with the Master Mix or testing procedure.

Data Interpretation

Leaf: Leaf results are determined using a single technical replicate. Each reaction is a result.

Seed: Seed results determined using a result set. For each sample, two technical replicates are used to determine the final assay result. MON 88302 seed result interpretation is as follows:

MON 88302 Result Set Interpretation (SEED ONLY)		
Replicate 1	Replicate 2	Final Result Interpretation
Not Detected (-)	Not Detected (-)	Negative
Not Detected (-)	Positive (+)	Positive
Positive (+)	Not Detected (-)	Positive
Positive (+)	Positive (+)	Positive

11. After the assay is complete, 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. **Do not open the tubes.**

12. To export results, return to the home screen, then "View Results." Insert a USB storage device into instrument (left side) and select each run to export and "Export Selected" and "OK." The results will be saved in a PDF summary report as well as .csv file format.

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