DNAble® Molecular Detection Kit for v1 and v2 *cp4 epsps*

Catalog No. DF-012 *Part #11977*

Highlights:

- *Molecular detection of v1 and v2* cp4 epsps *Soy*
- *Rapid amplification and detection in 15 minute assay*

Contents of DNAble Kit:

A. *2x RB1 Reaction Buffer*
B. cp4 epsps *Master Mix*
C. *Flat Caps*

Materials Not Provided:

- *Pipettes*
- *Pipette tips*
- *8-well AmpliFire Reader**
- *DNAble Extraction Set 5**

**available through EnviroLogix*

Intended Use

This test kit is intended for rapid qualitative detection of native and modified gene variants encoding *cp4 epsps* as expressed in first- (v1) and second- (v2) generation Roundup Ready® events in soybeans (GTS 40-3-2 [Roundup Ready] and MON89788 [Genuity® Roundup Ready 2 Yield™ and INTACTA RR2 PRO™] respectively).

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 *cp4 epsps* specific DNA in soybeans.

The amplified *cp4 epsps* specific DNA is detected and the results are displayed and interpreted at 15 minutes using our 8-well DNAble Reader.

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

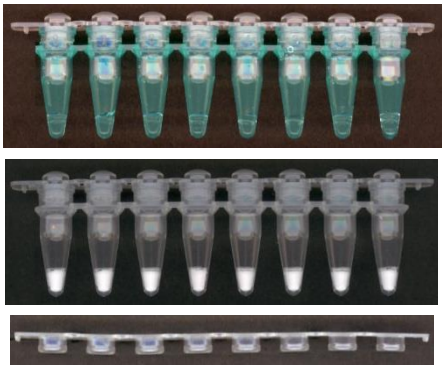
Important: Never open reaction tubes after reaction has occurred, as this will release amplified material into the environment and may contaminate subsequent reactions. Care should be taken when disposing of run reaction tubes to avoid possibility of tube leakage. Place completed reaction tubes back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

Kit Components

A. 2x RB1 Reaction Buffer: Provided in green 8-well strip tubes (6 strips)

B. *cp4 epsps* Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (6 strips). Each strip contains reagents to test four samples against both v1 RR and v2 RR.

C. 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

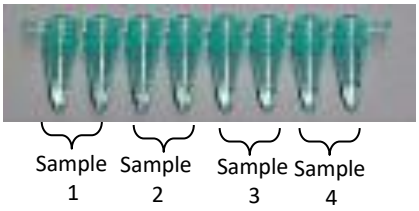
1. Follow Sample Extraction Set 5 product insert for sample preparation, extraction, and thermal denaturation.
2. Remove green Reaction Buffer strip tubes from the kit. Mark the left end tube to note orientation.

   ***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.*

3. Pipette **25 µL of diluted sample extract** (from Step 1) to the first two wells of the reaction buffer 8-well strip tube, using a fresh pipette tip for each replicate. Repeat for sample 2 in wells 3 and 4 and so on, using a fresh pipette tip for each transfer.
4. Recap tubes and tap down or centrifuge to ensure all liquid is at the bottom of the tube.

Step 3

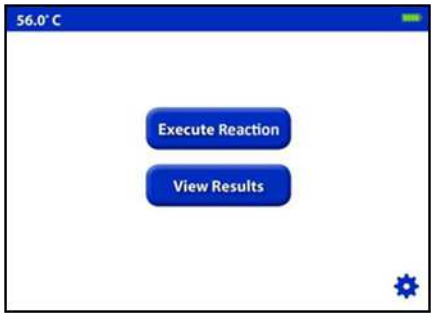
Transfer 25 µL diluted sample to three wells of buffer strip

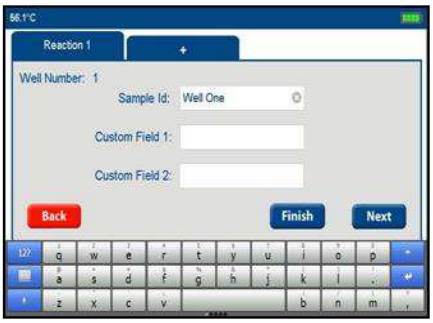


How to Run the DNAble Assay

DNAble assay protocol

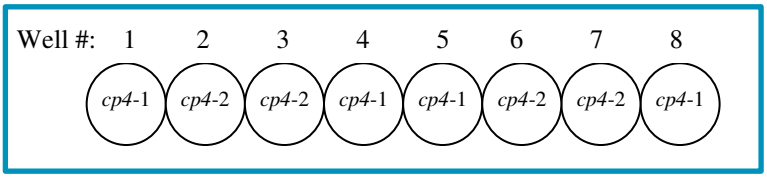
1. On the AmpliFire screen, select "**Execute Reaction**" then "**Scan Product Code**". Use the barcode on the Master Mix foil pouch to scan the *cp4 epsps* protocol on the 8-well AmpliFire Reader. **"DF-012#"** will display. Select "**Next**".
2. Under "Reaction Name" enter an appropriate reaction description**. This description is placed at the beginning of the file name.** Select "Next"
3. To enter sample specific information, add sample descriptions to the screens for Wells 1 through 8, clicking "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.



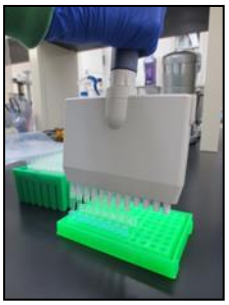


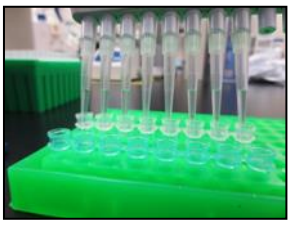
***Important:** Mark flat cap for orientation of the clear Master Mix Tubes (writing on clear tubes will interfere with results interpretation or leave marker residue in instrument).*

Each Strip contains enough Master Mix to test four samples. Layout is indicated below:



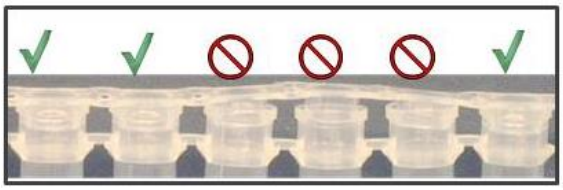
5. Using a multichannel pipette, transfer **50 µL from green Reaction Buffer Tubes (containing sample)** to clear Master Mix Tubes. Discard clear domed caps from green RB1 Buffer Tubes and Master Mix Tubes. Do **not** mix within the clear tube.





6. Cap Master Mix Tubes with provided **Flat Caps** strip. Mark the left end tube to note orientation.

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



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

    Important: The full assay time must be complete for complete result interpretation. (Empty wells will be interpreted as negative.)

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.

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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