DNAble® Molecular Detection Kit for Multi-Trait Testing

cp4 epsps, pat/pat & dmo

Catalog No. DF-041 *Part #12241*

Highlights:

- *Molecular detection of* cp4 epsps, pat/pat *and* dmo *Soybean*
- *Rapid amplification and detection in 15 minute assay*

Contents of DNAble Kit:

A. *2x RB1 Reaction Buffer*

B. cp4 epsps, pat/pat, *and* dmo *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 DNA of *cp4 epsps* as expressed in GTS 40-3-2 (Roundup Ready®); *cp4 epsps* as expressed in MON89788 (Genuity® Roundup Ready 2 Yield™ and INTACTA RR2 PRO™); *pat/pat* as expressed in various glufosinate resistant (LibertyLink®), and *dmo* as expressed in MON87708 (Genuity® Roundup Ready™ 2 Xtend™) soybeans, soybean meal, and soybean flakes.

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 *cp4 epsps*, *pat/pat*, and *dmo* specific DNA in soybeans and soybean meal.

The amplified *cp4 epsps*, *pat/pat*, and *dmo* specific DNA is detected in real-time and the results are displayed and interpreted within 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 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

A. 2x RB1 Reaction Buffer: Provided in green 8-well strip tubes (12)

B. *cp4 epsps*, *pat/pat*, and *dmo* Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (12 strips). Each strip contains reagents to test two samples against all four assays.

C. Flat Caps: used for capping the clear tubes prior to assay start (12 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.
- Pre-heat a dry heat block to 95°C. Allow heat block to warm for 30 minutes.

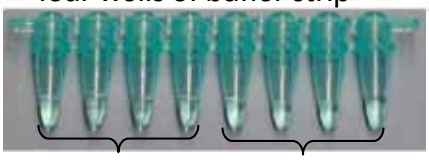
Sample Preparation

1. Follow Sample Extraction Set 5 product insert for sample preparation and extraction.
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 four wells of the reaction buffer 8-well strip tube, using a fresh pipette tip for each replicate. Repeat for sample 2 in wells 5-8, using a fresh pipette tip for each transfer.

Step 3

Transfer 25µL diluted sample to four wells of buffer strip



Sample 1 Sample 2

4. Recap tubes and tap down or centrifuge 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 Barcode**". Use the barcode on the master mix foil pouch to scan the protocol code on the 8-well AmpliFire Reader. **"DF-041#"** 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"

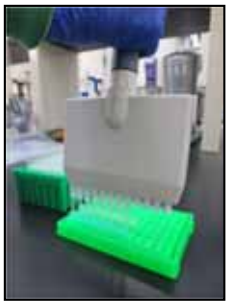
3. To enter sample specific information, add sample descriptions to the screens for Wells 1 through 8, clicking "Next" to advance to each Well. Select "Finish" to skip well-specific sample entry.

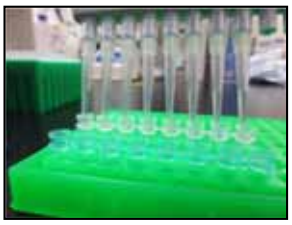
4. Remove clear Master Mix tubes from the foil pouch and gently tap down to ensure that the white pellet is at the bottom of the tubes.

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 two samples. Layout is indicated below:

Well #:	1	2	3	4	5	6	7	8
	RR1	RR2	LL	*dmo*	*dmo*	LL	RR2	RR1

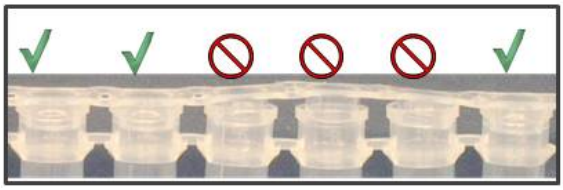




5. Using a multichannel pipette, transfer **50 µL from green strip tubes (containing sample)** to clear Master Mix tubes. Do **not** mix within the clear tube.

6. **Cap** Master Mix tubes with provided **Flat Caps** strip.

   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: Positive results may be interpreted prior to assay completion, but 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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