WHITE PAPER

Rapid Point-of-Need Molecular Detection of Roundup Ready 2® Positive Soybeans Using DNAble® Technology

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INTRODUCTION

Isothermal DNAble technology provides an innovative molecular testing solution for GM trait detection in processed material and for traits that are not detectable using traditional immunoassay methods. DNAble is a simple, rapid isothermal nucleic acid amplification method with results similar to PCR. However, unlike PCR, DNAble is highly effective using crude sample extracts that can be prepared at point-of-need in under 10 minutes. Furthermore, DNAble amplifies and detects specific target sequences in a non-laboratory setting with minimal equipment requirements, and at a fraction of the time and cost of PCR.

DNAble amplification chemistry uses a nicking enzyme and a strand displacing enzyme. These enzymes work together with dNTPs and specialized primers to achieve exponential DNA amplification of a target sequence in 15 minutes or less. Highly specific molecular beacons detect the amplified target and can be used for end-point detection or real-time quantitative results. Like PCR, DNAble can specifically detect genetic modifications in plants, but in a fraction of the time and in a point-of-need setting.

The DNAble® Molecular Detection Kit for v2 cp4 epsps from EnviroLogix (Cat. No. DF212) detects a sequence within the codon-optimized version of the aroA:CP4

gene expressing the CP4 EPSPS protein present in the event MON89788 (glyphosate-resistant Roundup Ready 2 Yield® (RR2), Roundup Ready 2 Xtend® Soy) in Soybean. The performance of this kit in ground soybean is described below with third-party validation data conducted by NSF International's Applied Research Center (NSF). Following a simple crude extraction protocol, the assay has a detection threshold of 0.5% RR2 with > 99% accuracy.

METHODS

Test Materials

Three separately manufactured kit lots of DNAble® Molecular Detection for v2 cp4 epsps (MON89788) in Soy (Cat. No. DF212) were used for validation testing. A single lot of DNAble® Extraction Set 5 (Cat. No. ACC-091) was used for all sample extractions.

Negative soybeans (conventional) used in these studies were sourced from Generon, Modena, Italy. Samples were confirmed negative for CP4 EPSPS

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by testing for GMO protein levels with EnviroLogix Lateral Flow Devices (LFD), but may contain trace GMO contaminants. Roundup Ready 2 Yield® (RR2) Trait Positive soybeans were commercially sourced. Whole soybeans (20 g) were ground in an Osterizer® blender using the Oster® Blender Mini-Blend® Jar with ice crushing blade and sealing ring. The whole soybeans were ground on the highest setting for 3, 20 second pulses with shaking to mix between each grind.

For cross-reactivity testing, pre-ground Certified Reference Materials (CRM) for non-RR2 GM soy (Roundup Ready® (RR1) expressing cp4 epsps; Liberty Link® expressing PAT-pat; Dicamba resistance (dmo) expressing dmo, and Cultivance® expressing csr1-2) were purchased from The European Commission Joint Research Centre Reference Materials Unit, Geel, Belgium or The American Oil Chemists' Society (AOCS), Urbana, IL. Note that the dmo certified reference material is dmo trait-only, not Roundup Ready 2 Xtend® (stacked with RR2).

For in-house testing at EnviroLogix, 40 individual mixes of 0.5% threshold samples were generated by adding 0.189 g of 100% cp4 epsps RR2 ground soybean to 37.651 g of conventional ground soybean. These weight values represent 1 positive soybean in 200 soybeans, based on average soybean weight to determine an accurate 0.5% threshold. The combined ground soybean blend was shaken well to mix.

For third-party assay validation at NSF International, 10 individual 0.5% threshold samples were generated by manually shaking 39.8 ± 0.1 g of conventional ground soybean for 30 seconds within a sterile 750 mL wide-mouth centrifuge bottle, then adding 0.20 ± 0.01 g of 100% cp4 epsps RR2-positive ground soybean. The bottles were thoroughly mixed for 5 minutes by alternating 30 second rounds of multidirectional manual shaking and vortexing on a multi-tube (platform head) benchtop vortex at maximum speed.

Sample Extraction

Samples were extracted at EnviroLogix and NSF International using DNAble® Extraction Set 5 (Cat. No. ACC-091) according to the product insert. In brief, 2 packed scoops of ground seed were added to 600 µL MB9 extraction buffer in a 2.0 mL extraction tube and heated at 95°C for 6 minutes on a dry heat block. The tube was then briefly vortexed and centrifuged at 10,000 x g for 3 minutes. The sample was then diluted by mixing 100 µL sample supernatant with 100 µL D4 buffer and vortexed to mix.

Assay Protocol

DNAble® Molecular Detection Kit for v2 cp4 epsps (MON89788) (Cat. No. DF212) assays were run according to the product insert. In brief, at standard room temperature (25°C), 25 µL of extracted sample was added to pre-aliquoted reaction buffer, then 50 µL of this diluted sample was added to the lyophilized master mix tubes. The assay tubes were capped, then amplified and read.

The assay runs at 56ºC on the AmpliFire®, which is a battery-powered portable isothermal amplification instrument. Each assay kit includes a barcode, which automatically runs the correct assay protocol on the instrument. The tubes are placed in the AmpliFire® unit and the assay is complete in under 15 minutes. Results are displayed on the AmpliFire® screen and may be exported via USB drive.

EnviroLogix Internal Validation Studies

For accuracy testing, 40 conventional and 40 0.5% RR2 ground seed samples were tested on three assay kit lots by two operators. Cross-reactivity against non-RR2 soy GMO ground seed standards was tested using one assay kit lot with ≥8 assay replicates. Assay performance at varying temperatures was tested in environmental chambers set to 18°C and 30°C at 40% humidity; 16 conventional and 16 0.5% RR2 ground seed samples were tested at each temperature.

NSF Independent Validation Studies

From a single conventional ground seed sample, 18 replicate sub-samples were prepared and tested across three assay kit lots (n=6 sub-samples per lot). From 10 independently blended 0.5% RR2 ground seed samples, 3 replicate sub-samples were prepared and tested across three assay kit lots (n=1 sub-samples per lot).

RESULTS

Both the internal and independent methods validation studies demonstrated >99% accuracy results from conventional ground soy and 100% sensitivity for 0.5% RR2 ground soy. Cross-reactivity internal testing with non-RR2 soy GMO standards showed 100% specificity. When sample extraction and assay protocols were run at 18°C, the assay demonstrated 94% accuracy for conventional ground soy samples and 100% sensitivity for 0.5% RR2 ground soy samples. At 30°C, the assay demonstrated 100% accuracy for conventional ground soy samples and 100% sensitivity for 0.5% RR2 ground soy samples. It should be noted that the conventional ground soy used may have trace GMO contamination that might account for the accuracy results observed.

Internal Accuracy Data

Ground seed	DNAble result (positives)			Total % positive
	Kit lot #1	Kit lot #2	Kit lot #3	
Conventional	0/40	1/40	0/40	<1%
0.5% RR2	40/40	40/40	40/40	100%

NSF Independent Accuracy Data

Ground seed	DNAble result (positives)			Total % positive
	Kit lot #1	Kit lot #2	Kit lot #3	
Conventional	0/6	0/6	0/6	0%
0.5% RR2	10/10	10/10	10/10	100%

Cross-reactivity Data

Certified reference material	DNAble result (% positive)
10% RR1	0/12 (0%)
10% Lib. Link	0/12 (0%)
100% dmo	0/8 (0%)
100% Cultivance	0/8 (0%)

Temperature Variation Data

Ground seed	DNAble result (positives)		Total % positive
	18°C	30°C	
Conventional	1/16	0/16	3%
0.5% RR2	16/16	16/16	100%

CONCLUSION

The EnviroLogix DNAble® Molecular Detection Kit for v2 cp4 epsps (MON89788) in Soy performed with 100% sensitivity to the Roundup Ready 2 Yield trait at a 0.5% qualitative threshold and >99% accuracy for non-RR2 soy GMO traits and presumed trait-negative soybeans in both internal and external validations. This assay is easy to use, rapid (total time to result <30 min), and may be deployed in the field with minimal equipment and training requirements. DNAble technology provides a cost-effective, unmatched combination of immunoassay-style ease-of-use with highly specific molecular diagnostic results similar to PCR.

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