DNAble®
Sample Extraction Set 9

Catalog No. ACC-109 *Part# 12501*

Set Contains:

- *MB13 Extraction Buffer*
- *D6 Dilution Buffer*
- *50 mL clear conical tubes (50) for extraction*
- *1.5 mL micro-centrifuge tubes (50) for centrifugation*

Materials Not Provided:

- *Oster Blender and cup*
- *Clean 50 mL conical tubes or 10 mL serological pipettes for measuring buffers*
- *Pipette helper or bulb*
- *Precision pipette(s) capable of delivering 10-1000 µL*
- *Filtered pipette tips (P20 and P200)*
- *Scoopula(s)*
- *Dry heat block capable of 100±1.5°C, with insert suitable for 50 mL tubes*
- *Vortex*
- *Centrifuge*
- *Timer*

Intended Use

This Set provides for extraction for the detection of DNA from ground soybean when used with DNAble Kit for Molecular Detection of MON87705 Event in Soybean, Catalog No. DF-009 (Part #12493).

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.

How the Kit Works

Ground soybean is added to a 50 mL tube followed by MB13 Extraction Buffer. The sample is heated to enable extraction of DNA. D6 Dilution Buffer is then added followed by a centrifugation step. The supernatant is then used in the DNAble reaction.

Precautions and Notes

DNAble is a highly sensitive assay; therefore, the following precautions are recommended to reduce the chance of sample contamination:

- Separated work areas are recommended for each of the following:
  - DNAble sample preparation
  - DNAble amplification and detection
- Clean the work station and pipettes with 10% bleach before and after use
- Do not reuse kit disposables
- Wash scoopulas used for ground soy in 10% bleach and ensure the scoop is dry before reusing
- Change pipette tips in between samples, <u>including replicates</u> from the same sample extract
- Wear gloves and change between handling of samples
- Avoid delays between sample preparation steps and between sample preparation and DNA amplification
- MB13 Extraction Buffer and D6 Dilution Buffer are stable for 1 year post manufacture when stored refrigerated (2-8°C)

Sample Preparation and Extraction

1. Pre-heat the dry heat block to **100°C.** Allow heat block to warm for 1 hour. Verify heat block is holding temperature with ±1.5°C using a simple thermometer.
2. Remove buffers from refrigerator and allow to come to room temperature while the heat block warms.
3. Follow below protocol for processing soybean:
   a. Weigh out 200 whole soybeans into small sized Oster Blender cup (ensure that rubber gasket is in place and lid is closed tightly).
   b. Grind on highest speed setting for 20 seconds, then shake down cup.
   c. Repeat 20-second grind twice more (for a total of 3 repetitions) shaking down between each 20-second grind.
   d. Using a clean scoopula, carefully scoop ground soybean into a clean 50 mL tube until it reaches the 5 mL line. Do not tap/pack down; this is an approximation.
   e. Using a clean 50 mL conical tube to measure buffer volume (or a 10 mL serological pipette), add 10 mL of MB13 Extraction Buffer to ground soy, cap and vortex for 5 seconds.

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f. Heat at 100°C, in the pre-heated 50 mL heat block, for 12 minutes.

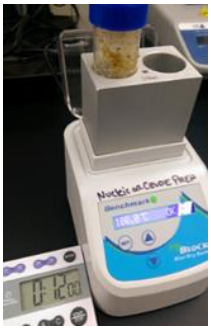
g. Carefully remove from heat and using a clean 50 mL conical tube (or a 10 mL serological pipette) add 10 mL of D6 Dilution Buffer.

h. Vortex for 5 seconds AND then shake for 5 seconds.

i. Transfer an aliquot of approximately 1 mL extract to a 1.5 mL micro-centrifuge tube.

j. Centrifuge extract for 1 minute at 2000 x g.

k. 10 µL of this diluted crude extract will be used in the subsequent DNAble reaction. (DNAble Kit, Sample Preparation Step 3).

1. Grind Soybeans Oster Blender on high for 3 cycles of 20 seconds	 **2.** Add ground soy to **5 mL line**	 **3.** Add **10 mL** Extraction Buffer **4. Vortex 5 seconds** on high setting	 **5.** Heat for **12 minutes @ 100°C** **6. Remove from heat**	 **7.** Add **10 mL Dilution Buffer; vortex 5 seconds,** then **shake 5 seconds**	**8. Transfer** up to 1 mL to 1.5 mL tube **9. Centrifuge** 1 minute @ 2000 x *g* **10.** Use **10 µL extract** in DNAble reaction

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