# DNAble® Sample Extraction Set 7

***Set Contains:***

- *MB4 Extraction Buffer*
- *D4 Dilution Buffer*
- *2.0 mL clear micro-centrifuge tubes (50) for extraction*
- *1.5 mL blue micro-centrifuge tubes (50) for dilution*
- *Grain scoops (8)*

***Materials Not Provided:***

- *Dry heat block capable of 95±1°C, with insert suitable for 2 mL tubes*
- *Vortex*
- *Micro-centrifuge capable of 10,000 x* g
- *Timer*
- *Pipettes*
- *Pipette tips*

Catalog No. ACC-101

## Intended Use

This Set provides for extraction for the detection of DNA from ground cottonseed when used in combination with the following DNAble Kits: Molecular Detection Kit for MON88701 Event (*dmo*) in cottonseed (Cat. No. DF-150, Part #12396).

Contact Technical Support for questions regarding any other matrix types or deviations from the protocol.

## 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

An aliquot of MB4 buffer is added to a micro-centrifuge tube followed by two packed scoops of ground cottonseed. The sample is heated to enable the extraction of DNA and is then followed by a centrifugation step. Sample is transferred to a new tube where it is then diluted with D4 dilution buffer. This sample will be 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
- Grain scoops are reusable: wash 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
- MB4 is stable for 1 year post manufacture when stored refrigerated (2-8°C)

## Sample Preparation and Extraction

1. Pre-heat a dry heat block to 95°C.

   *Allow heat block to warm for a minimum of 30 minutes. Verify heat block is holding temperature with ±1.5°C using a simple thermometer.

2. Grind 200 cottonseeds in a Waring blender for 20 seconds on high and tap jar to ensure even grinding. Repeat 1 additional time for total of 2 grinds.
3. Using a pipette and a clean tip, add 600µl of MB4 extraction buffer to a 2.0 mL clear extraction tube (1 tube per sample).
4. Using a clean grain scoop*, add **two packed** level scoops of ground cottonseed to extraction tube with buffer, then cap tightly.

   *use a clean scoop for each grind to avoid contamination of samples – see instructions in Precautions & Notes

5. Vortex to ensure complete suspension of sample.
6. Heat tube from previous step at 95°C for 10 minutes (±30 seconds).

7. Remove the 2 mL extraction tube from heat block and vortex to mix.
8. Centrifuge sample at 10,000 x *g* for 3 minutes (±30 seconds).
9. Add 150 µl of D4 dilution buffer to a blue 1.5 mL dilution tube. One tube per sample.
10. Transfer 50 µl of supernatant from the middle of the extract to the 1.5 mL blue dilution tube containing dilution buffer and vortex to mix.

    *Important: Take care to avoid the lipid layer at the top and the settled cottonseed at the bottom. This step should occur immediately after centrifugation.*
11. 25 µL of this diluted crude extract will be used in the subsequent DNAble reaction (DNAble Kit DF-150, Sample Preparation Step 3).

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