ENVIROLOGIX

DNAble®

Sample Extraction Set 5

Set Contains:

- *MB9 Extraction Buffer*
- *D4 Dilution Buffer*
- *2.0 mL clear micro-centrifuge tubes (25) for extraction*
- *1.5 mL blue micro-centrifuge tubes (25) for dilution*
- *Grain scoops (4)*

Materials Not Provided:

- *Precision pipette(s) capable of delivering 25-1000 µL*
- *Pipette tips*
- *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*

Catalog No. ACC-091 *Part #12038*

Intended Use

This Set provides for extraction for the detection of DNA from ground soybean and canola when used in combination with the following DNAble Kits:

	Matrix	Catalog No.	Part #
Multi-Trait (v1/v2 *cp4 epsps* & *pat/pat*)	Soybean	DF-031	11979
Multi-Trait (v1/v2 *cp4 epsps*, *pat/pat* & *dmo*)	Soybean	DF-041	12241
dmo (DMO)	Soybean	DF-050	12242
pat/pat	Soybean	DF-014	11978
cp4 epsps (v1 & v2)	Soybean	DF-012	11977
cp4 epsps (v1)	Soybean	DF-112	11976
cp4 epsps (v2)	Soybean	DF-212	12129
MON 88302 Canola Leaf and Seed	Canola	DF-017	12771

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 MB9 buffer is added to a micro-centrifuge tube followed by ground soybean, ground canola, or canola leaf. The sample is heated to enable the extraction of DNA. A centrifugation and dilution step follows.

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, including replicates 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
- MB9 is stable for 1 year post manufacture when stored refrigerated (2-8°C)

Sample Preparation and Extraction

1. Pre-heat a dry heat block
   - 95°C for bulk soybeans
   - 85°C for canola bulk seed or leaf

   Allow heat block to warm for 30 minutes. Using a thermometer, verify heat block temperature is ±1.5°C.
2. Choose protocol based on sample type:

a. Soybeans

i. Grind a minimum of **200 soybeans** (as determined by average seed weight) in an Oster blender for 20 seconds. Shake down the cup. Repeat 2 additional times (shaking down cup between grinds) for a total of 3 grinds.

ii. **IMPORTANT: Shake MB9 bottle for 5 seconds before <u>each</u> dispense**. This is to ensure that undissolved particulate matter is homogenously distributed in the buffer.

iii. Add **600 µL MB9** Extraction Buffer to 2.0 mL clear extraction tube (1 tube per sample).

iv. Using a clean grain scoop*, add **2 packed** level scoops of ground soybean to extraction tube with buffer, then cap tightly. Flick tube to ensure complete suspension of sample. *use a clean scoop for each grind to avoid contamination of samples – see instructions in Precautions & Notes

v. Heat tube from previous step at **95°C for 6 minutes** (±30 seconds).

vi. Remove the 2 mL extraction tube from heat block and vortex 5 seconds to mix.

vii. Centrifuge sample at 10,000 x *g* for 3 minutes (±30 seconds).

viii. Add **100 µL D4 Buffer** to a clean 1.5 mL blue dilution tube. Add **100 µL supernatant** from centrifuged sample, taking care to avoid settled soybean. Vortex to mix.

ix. **25 µL** of this diluted crude extract will be used in the subsequent DNAble reaction (DNAble Kit Product Insert, Sample Preparation Step 3).

b. Canola Seed

i. Grind a minimum of **2000 canola seeds** (as determined by average seed weight) in an Oster blender for 20 seconds. Shake and tap the cup to dislodge any unground material. Repeat the 20 second grind 2 additional times (shaking between grinds). Each sample will have a total of 3 (20 second) grinds.

ii. **IMPORTANT: MB9 bottle must be shaken for 5 seconds before <u>each</u> dispense**. This is to ensure that undissolved particulate matter is homogenously distributed in the buffer.

iii. Add **600 µL MB9** Extraction Buffer to 2.0 mL clear extraction tube (1 tube per sample).

iv. Using a clean grain scoop*, add **2 packed** level scoops of ground canola to extraction tube with buffer, then cap tightly. Flick tube to ensure complete suspension of sample. *use a clean scoop for each grind to avoid contamination of samples – see instructions in Precautions & Notes

v. Heat tube from step iv. at **85°C for 6 minutes** (±30 seconds).

vi. Remove the 2 mL extraction tube from heat block and vortex.

vii. Centrifuge sample at 10,000 x *g* for 3 minutes (±30 seconds).

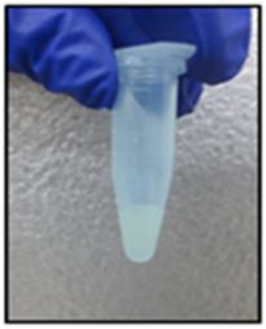
viii. Add **200 µL D4** Buffer to a clean 1.5 mL blue dilution tube. Add **50 µL supernatant** from centrifuged sample, taking care to avoid settled canola. Vortex to mix.

ix. **Two 25 µL aliquots** of this diluted crude extract will be used in the subsequent DNAble reaction (DNAble Kit, Sample Preparation Step 3).

iv. Add grain to MB9

v. Heat

viii. Final dilution



c. Canola Leaf

i. **IMPORTANT: MB9 bottle must be shaken for 5 seconds before each dispense**. This is to ensure that undissolved particulate matter is homogenously distributed in the buffer.

ii. Add **600 µL MB9** to 2 mL clear 2.0 mL clear extraction tube.

iii. Collect one leaf punch by capping the extraction tube around a young canola leaf.

iv. Clear away any excess leaf tissue.

v. Submerge the leaf punch in the buffer using a toothpick or clean pipette tip.

vi. Heat leaf punch at **85°C for 6 minutes** (±30 seconds).

vii. Remove the 2 mL extraction tube from heat block and vortex.

viii. Open tube cap and place in a rack. **Leave OPEN tube in rack undisturbed for 1 minute** to allow buffer particulates to settle.

ix. **25 µL** of this crude extract will be used in the subsequent DNAble reaction (DNAble Kit, Sample Preparation Step 3). *

*** IMPORTANT: TUBE SHOULD BE LEFT UNDISTURBED IN RACK PRIOR TO AND DURING PIPETTING.** Carefully aspirate 25 µL from just below the surface of the extract taking care not to disturb the buffer particles that have settled at the bottom of the tube.

iii. Leaf sample collection

LIMITED WARRANTY

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