

**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  $\mu$ L
- Pipette tips
- Dry heat block capable of  $95 \pm 1^\circ\text{C}$ , 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 #	Part #
Multi-Trait (RR1, RR2, LL)	Soybean	DF-031	11979
Multi-Trait (RR1, RR2, LL, DMO)	Soybean	DF-041	12241
<i>dmo</i> (DMO)	Soybean	DF-050	12242
<i>pat/pat</i> (LL)	Soybean	DF-014	11978
<i>cp4 epsps</i> (RR1 and RR2)	Soybean	DF-012	11977
<i>cp4 epsps</i> (RR1, GTS 40-3-2)	Soybean	DF-112	11976
<i>cp4 epsps</i> (RR2, MON89788)	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](http://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 ( $2-8^\circ\text{C}$ )

**Sample Preparation and Extraction**

1. Pre-heat a dry heat block
  - $95^\circ\text{C}$  for bulk soybeans
  - $85^\circ\text{C}$  for canola bulk seed or leaf

Allow heat block to warm for 30 minutes. Using a thermometer, verify heat block temperature is  $\pm 1.5^\circ\text{C}$ .

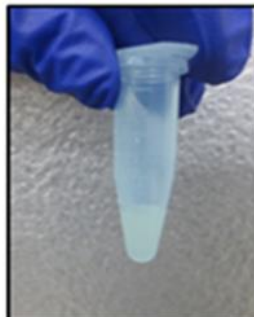
2. Choose protocol based on sample type:

**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 each 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** ( $\pm 30$  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 ( $\pm 30$  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 each 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** ( $\pm 30$  seconds).
- vi. Remove the 2 mL extraction tube from heat block and vortex.
- vii. Centrifuge sample at 10,000 x g for 3 minutes ( $\pm 30$  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  $\mu$ L 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** ( $\pm$ 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  $\mu$ L** 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  $\mu$ L 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**

For Technical Support  
Contact Us At:

**EnviroLogix**

500 Riverside Industrial Parkway  
Portland, ME 04103-1486 USA

**Tel: (207) 797-0300**

**Toll Free: 866-408-4597**

**Fax: (207) 797-7533**

e-mail: [dnable@envirologix.com](mailto:dnable@envirologix.com)

Website: [www.envirologix.com](http://www.envirologix.com)

EnviroLogix Inc. (“EnviroLogix”) warrants the products sold hereunder (“the Products”) against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. If the Products do not conform to this Limited Warranty and the customer notifies EnviroLogix in writing of such defects during the warranty period, including an offer by the customer to return the Products to EnviroLogix for evaluation, EnviroLogix will repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period.

ENVIROLOGIX MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of EnviroLogix products appearing in EnviroLogix published catalogues and product literatures are EnviroLogix’ sole representations concerning the Products and warranty. No other statements or representations, written or oral, by EnviroLogix’ employees, agents or representatives, except written statements signed by a duly authorized officer of EnviroLogix Inc., are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

EnviroLogix does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by EnviroLogix, or against damages resulting from such non-EnviroLogix made products or components. EnviroLogix passes on to customer the warranty it received (if

any) from the maker thereof of such non-EnviroLogix made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by EnviroLogix.

**THIS WARRANTY IS EXCLUSIVE.** The sole and exclusive obligation of EnviroLogix shall be to repair or replace the defective Products in the manner and for the period provided above. EnviroLogix shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall EnviroLogix be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of EnviroLogix with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

*EnviroLogix, the EnviroLogix logo, DNABLE, and the DNABLE logo are trademarks of EnviroLogix Inc.*

© EnviroLogix 2018