DNAble® Detection Kit for *Listeria monocytogenes*

Catalog No. DF-019 *Part #12135*

Highlights:

- *Molecular Detection of* Listeria monocytogenes
- *Rapid amplification and detection in 15 minute assay*

Contents of DNAble Kit:

- *RB1 Reaction Buffer*
- Listeria monocytogenes *Master Mix*
- *Flat caps*

Materials Not Provided:

- *Sample Extraction Set 3 (Cat# ACC-089)*
- *Oxoid Novel Enrichment (ONE) broth*
- *Precision pipette capable of delivering 5 µL*
- *Precision multi-channel pipette capable of delivering 50 µL*
- *Pipette tips*
- *DNAble Reader**
- *Laptop* (optional)*
- *Optional: DNAble* Listeria *Positive Control* (Cat. No. ACC-119)*

** available through EnviroLogix*

Intended Use

This test kit is intended for the qualitative detection of *Listeria monocytogenes* DNA as an indication of *Listeria monocytogenes* presence. Samples should be enriched in Oxoid Novel Enrichment (ONE) broth and prepared for DNA amplification using Extraction Set 3 (Cat. No. ACC-089, Part #11991) before using this Detection Kit.

Intended User

The DNAble *Listeria monocytogenes* assay is designed to be simple and user friendly. It is designed for use by personnel with appropriate training in handling human pathogens, and in Microbiology and Molecular Assay techniques. Training specific to the DNAble *Listeria monocytogenes* assay will be provided by EnviroLogix; contact Technical Service or visit envirologix.com/DNAble for more information.

Test Principle

Three products are used to enable qualitative *Listeria monocytogenes* DNA detection. Oxoid Novel Enrichment (ONE) broth is used to facilitate growth of *Listeria* organisms. DNAble Sample Extraction Set 3 includes reagents and protocols for sample preparation. The DNAble Molecular Detection Kit for *Listeria monocytogenes* contains lyophilized reagents for DNA amplification and detection arrayed in 8 well strips. DNAble is an isothermal technology enabling rapid amplification of a specific DNA target. Portions of cultured and prepared sample are added to a reaction buffer. This mixture is transferred to the lyophilized master mix, containing all the reagents needed to specifically recognize, amplify and detect a *Listeria monocytogenes* specific DNA sequence. The amplified *Listeria monocytogenes* specific DNA is detected in real-time and the results are displayed and interpreted within 15 minutes using the DNAble Reader.

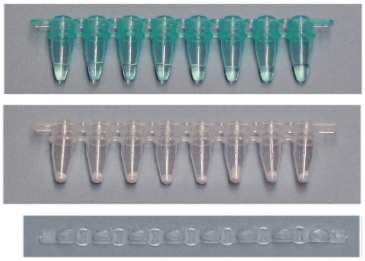
Precautions and Notes

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 culture
  - DNAble sample preparation
  - DNAble amplification and detection
- **Important: Never open reaction tubes after reaction has occurred, as this will release amplified material into the environment and may contaminate subsequent reactions.**
- Clean the work station and pipettes with 10% bleach before and after use.
- Do not reuse kit disposables
- Change pipette tips in between samples
- Wear gloves and change between handling of samples
- Avoid delays between sample preparation steps and between sample preparation and DNA amplification.
- The kit may be stored refrigerated at 4-8°C up to 6 months past the manufacture date. See expiration date on kit box label.
- **Controls:** *Listeria monocyotogenes* Amplification Positive Control is available as an accessory, Cat. No. ACC-119, to enable user verification of performance. Instructions on the use of this positive control, and optional use of DNAble extraction buffer as a negative control, are included with this accessory kit.

- Results may be confirmed using procedures defined in the US FDA the Bacteriological Analytical manual, BAM, (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm).
- **Safety:** *Listeria monocytogenes* is pathogenic and classified as a Biosafety Level 2 organism. Personnel should be appropriately trained and should use personal protective equipment. Laboratories should follow appropriate local safety and environmental regulations and guidelines for containment and disposal as described in the Center for Disease Control and Prevention Manual, "Biosafety in Microbiological and Biomedical Laboratories" (http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf).

Kit Components



A. RB1 Reaction Buffer: Provided in green 8-well strip tubes (6 per kit)

B. *Listeria monocytogenes* Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (6 per kit)

C. Flat Caps: used for capping the clear tubes prior to assay start (6 per kit)

How to Run the DNAble Amplification Assay

Note: ***Before beginning assay, extract sample(s) according to sample preparation instructions included with Sample Extraction Set.***

Before Testing

1. Remove needed DNAble Detection Kit reagents from refrigerated (4-8°C) storage. Allow reagents to come to room temperature (22-26°C) before opening sealed white pouch.
2. Turn on the DNAble Reader in remote mode and connect to the computer (see Reader Instructions).
3. Important: Remove green RB1 Reaction Buffer tubes (A) from bag and tap down to ensure that the liquid is at the bottom of the tube.
4. Add 5 µL of extracted sample (Step 13 of extraction set 3) to RB1 Reaction Buffer (Green Tube). Recap with green strip caps and gently flick tubes to mix sample with buffer.
5. To Run a Test, Press "Run" and select "Listeria mono_v1" from the drop down menu.
6. Correctly label the User Name, Lot ID (DNAble Kit Lot), and Sample ID fields in the software **prior** to starting the assay workflow.

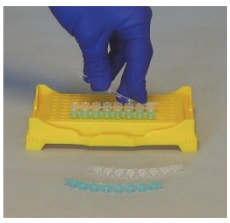
DNAble assay protocol

7. Ensure that steps 1-6 from above have been performed and that all assay reagents, multichannel pipette and tips, and flat caps are ready for use. One or two 8-well strips (8 or 16 tests) may be performed at a time.

   Set up Master Mix strip tube and green Reaction Buffer strip tube so that pipet transfer step occurs from front to back (not side-to-side or back-to-front). This will help avoid cross-contamination of samples.

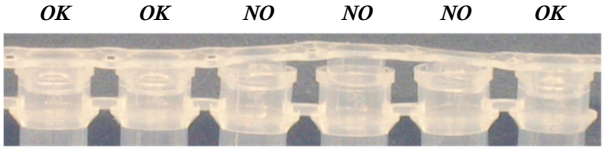
   Important: *Gently tap down the Master Mix tubes to ensure that the white lyophilized pellet is at the bottom of the tubes.*

8. Using a multichannel pipette transfer **50 µL** from **green Reaction Buffer Tubes** (5 µL of Extracted Sample + Reaction Buffer) to the **clear Master Mix Tubes**.



9. Re-cap with "Flat Caps" provided. Quickly repeat Steps 8 and 9 for a second 8-well strip if 16 tests will be performed. Mark one side of flat cap with marker for orientation – do not label sides of tubes.

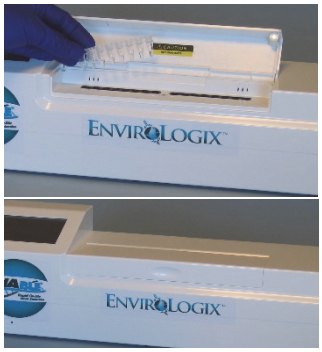
Important: *Ensure that tubes are completely sealed with flat caps*



Important: *Ensure that any bubbles that may exist in the bottom of the tubes are removed by gently tapping or flicking the tubes.*

10. Quickly place the ***clear resuspended*** **Master Mix** strip tubes into the DNAble Reader.

11. Close lid of DNAble Reader and immediately **click "Start"** on software.

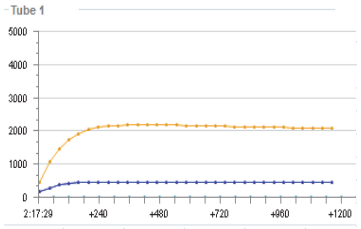


Important: *It is critical to perform this step as soon as possible after placing the re-suspended Master Mix tubes in the instrument.*

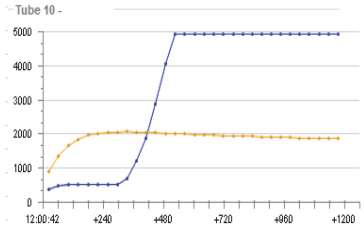
12. After 15 minute assay time, the software will display results as negative (-), positive (+), or invalid (?).

Important: *Results will be interpreted only after the assay is complete. Typically there is a short delay between completion of the assay and display of results. Do not initiate a new assay until results are displayed. An invalid result is generated if the well is empty, if the sample has excessively high background signal (often indicative of incorrect sample processing), or internal control curve varied from the normal (can be an indication of amplification inhibition or delay in starting the amplification reaction). Users are advised to repeat the analyses starting with sample transfer to RB1 buffer through amplification and detection. Contact EnviroLogix Technical Customer Support for further questions.*

Following are the examples of the amplification curves pertaining to the valid negative and valid positive on T16 DNAble Reader.



Valid Negative



Valid Positive

13. Gently remove the completed reaction tubes from the DNAble Reader and place back in original zippered pouch prior to disposal. **Do not open tubes**.

14. The assay data file (.json) may be saved with a user-defined name using the "Save As" function. A results summary including the sample ID and amplification graphs is also available using the Print function.

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