DNAble® Molecular Detection Kit for STECs

Highlights:

- *Molecular Detection of Shiga toxin producing* E. coli *(STECs)*
- *Rapid amplification and detection in 15 minute assay*

Contents of DNAble Kit:

- *RB1 Reaction Buffer (12 strips)*
- stx1 *Master Mix (6 strips)*
- stx2 *Master Mix (6 strips)*
- *Flat caps (12 strips)*

Materials Not Provided:

- *Sample Extraction Set 4 (Cat. No. ACC-090)*
- *Precision pipette capable of delivering 5 µL*
- *Precision multi-channel pipette capable of delivering 50 µL*
- *Pipette tips*
- *DNAble Reader T16**
- *Laptop* (optional)*

** available through EnviroLogix*

Catalog No. DF-042 *Part #11765*

Intended Use

This test kit is intended for the qualitative detection of shiga-toxin DNA (*stx1, stx2*) as an indication of Shiga toxin producing *Escherichia coli* (STECs) presence. Samples should be enriched in modified tryptic soy broth (mTSB), using 8 µg/mL Novobiocin and prepared for DNA amplification using the appropriate *E. coli* Sample Extraction Set 4 (Cat. No. ACC-090) before using this detection kit.

Intended User

The DNAble STECs 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 STECs assay will be provided by EnviroLogix; contact Technical Service or visit envirologix.com for more information.

Test Principle

Two separate lyophilized master mix are used to enable qualitative *stx1* and *stx2* DNA detection. DNAble Sample Extraction Set 4 includes reagents and protocols for sample preparation. The DNAble Molecular Detection Kit for STECs contains lyophilized reagents for isothermal *stx1* and *stx2* DNA amplification and reaction buffer 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 *stx1* or *stx2* specific DNA sequence for STECs. These amplified STECs-specific DNA are detected in real-time and the results are displayed and interpreted within 15 minutes using the DNAble Reader T16.

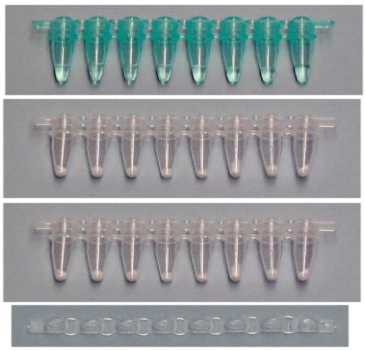
Precautions and Notes

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:
  - Microbial 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.

- Results may be confirmed using procedures defined in the USDA-MLG (http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/foodborne-illness-and-disease/escherichia-coli-o157h7/ct_index)
- **Safety:** STECs are pathogenic and are 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 (12 per kit)

B. *stx1* Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (6 per kit)

C. *stx2* Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (6 per kit)

D. Flat Caps: used for capping the clear tubes prior to assay start (12 per kit)

How to Run the DNAble Amplification Assay

Note: *Before beginning assay, extract sample(s) according to sample preparation instructions included with each Sample Extraction Set. (Cat. No. ACC-090)*

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 to two strips of 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 "*DF-042_rev030615*" 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: one for *stx1* and the other for *stx2*

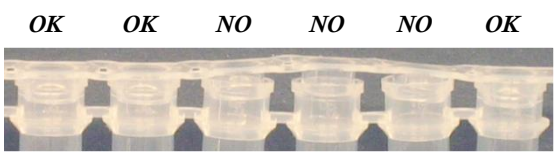
   Set up Master Mix strip tube and green Reaction Buffer strip tube so that pipette transfer step occurs from front to back (not side-to-side or back-to-front). This will help avoid cross-contamination of samples.

   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 *stx1* clear Master Mix Tubes.

9. Using a multichannel pipette transfer 50 µL from green Reaction Buffer Tubes (5 µL of Extracted Sample + Reaction Buffer) to the *stx2* clear Master Mix Tubes.
10. 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 the 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.*

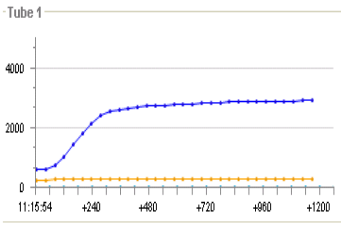
11. Quickly place the ***clear suspended*** **Master Mix** strip tubes into the DNAble Reader.
12. Close lid of DNAble Reader and immediately **click "Start"** on software.

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

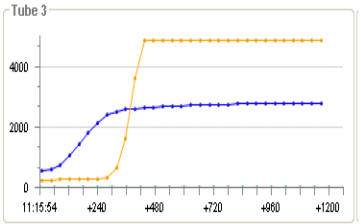
13. After 15 minute assay time, the software will display results as negative (-), positive (+), or invalid (?).

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 DNAble Reader T16.



Valid Negative



Valid Positive

14. Gently remove the completed reaction tubes from the DNAble Reader and place back in original zippered pouch prior to disposal. **Do not open tubes**.
15. 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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