# DNAble® Molecular Detection Kit for *Salmonella* Plus

***Highlights:***
- *Molecular Detection of Salmonella*
- *Rapid amplification and detection in 15 minute assay*

***Contents of DNAble Kit:***
- *RB1 Reaction Buffer*
- *Salmonella Master Mix*
- *Flat Caps*
- *RB1 in vial*

***Materials Not Provided:***
- *Sample Extraction Set* (Cat# ACC-085 and ACC-089)*
- *Salmonella Supplement* (Cat# XSALMD550-300)*
- *Precision pipette capable of delivering 5 µL*
- *Precision multi-channel pipette capable of delivering 50 µL*
- *Pipette tips*
- *DNAble Reader**
- *Laptop* (optional)*
- *Optional: DNAble* Salmonella *Positive Control* (ACC-226)*

** available through EnviroLogix*

Catalog No. DF-126 *Part #12010*

## Intended Use

This test kit is intended for the qualitative detection of *Salmonella* DNA as an indication of *Salmonella* presence. Samples should be enriched in 2% Modified Buffered Peptone Water (mBPW), using DNAble *Salmonella* Media Supplement (Cat# XSALMD550-300) and prepared for DNA amplification using the appropriate *Salmonella* Extraction Set (Cat# ACC-085 or ACC-089) before using this Detection Kit.

## Intended User

The DNAble *Salmonella* Plus 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 *Salmonella* Plus assay will be provided by EnviroLogix; contact Technical Service or visit envirologix.com/salmonella for more information.

## Test Principle

Three products are used to enable qualitative *Salmonella* DNA detection. DNAble *Salmonella* Media Supplement provides a selective agent and components that facilitate growth of stressed *Salmonella* organisms. DNAble Sample Extraction Sets include reagents and protocols for sample preparation. The DNAble Molecular Detection Kit for *Salmonella* Plus contains lyophilized reagents for isothermal 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 extract are added to a reaction buffer and heat denatured. This mixture is transferred to the lyophilized Master Mix, containing all the reagents needed to specifically recognize, amplify and detect a *Salmonella* specific DNA sequence. The amplified *Salmonella*-specific DNA is detected and the results are displayed and interpreted at 15 minutes using the DNAble Reader.

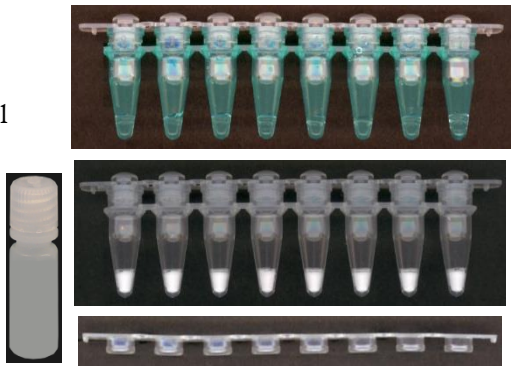
## Precautions and Notes

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.
- Additional RB1 Reaction Buffer is provided in the event that any volume is lost from the green strip tubes. Visually inspect strip tubes and if any appear to have lost volume, pipette out any remaining RB1 from deficient wells and replace with 50 µL of RB1 from the vial.
- 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:** *Salmonella* Amplification Positive Control is available as an accessory, Part #11768, 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:** *Salmonella* is pathogenic and is 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.** *Salmonella* 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)

**D.** Additional RB1: Provided in a vial (see Precautions and Notes)

## 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.***

**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. Important: Remove green RB1 Reaction Buffer tubes (A) from bag and ensure that the entire buffer volume is at the bottom of the tubes; either centrifuge the green strip, or while holding the strip, use a downward flick of the wrist. Then visually inspect the tubes to ensure equal volumes of buffer in each. If any of the vials appears to have lost volume during shipping, see Precautions and Notes for steps to take.
3. Add 5 µL of extracted sample to RB1 Reaction Buffer (Green Tube). Recap with clear domed strip caps and gently flick tubes to mix sample with buffer.
4. Start up and prepare DNAble Reader for testing including test code, User Name, Lot ID, etc. prior to initiating work flow.
5. Assure all assay reagents, multichannel pipette and tips, and Flat Caps are ready for use. 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.*

   ***Important:*** *Label tube for orientation at the top of the tube (writing on the bottom half of the tube will interfere with results interpretation).*
6. Prepare DNAble Reader:

| DNAble T16 Reader | AmpliFire Reader |
|---|---|
| • Turn on the DNAble Reader in remote mode and connect to the computer (see Reader Instructions).<br>• To prepare the machine, Press "Run" and select "*SalmonellaPlus_v3*" from the drop down menu.<br>• Correctly label the User Name, Lot ID (DNAble Kit Lot), and Sample ID fields in the software prior to starting the assay workflow. | • Turn the instrument "ON" using a switch on the right panel of the instrument.<br>• On the screen, select "Execute Reaction" then "Scan Product Code". Use QR code provided on the Master Mix foil pouch to scan the *Salmonella* Plus protocol on the AmpliFire Reader. "Sal_Plus_(lot#)" will display. Select "Next".<br>• Under "Reaction Name" enter the run information. Select "Next" and then "Finish" to skip well-specific sample entry if desired. |

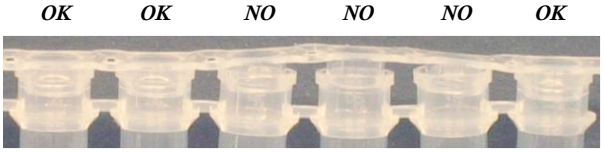
**DNAble assay protocol:**

7. 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**. Discard clear domed caps from green RB1 Buffer Tubes and Master Mix Tubes.

8. Re-cap with "Flat Caps" provided. (Quickly repeat Steps 7 and 8 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.*




9. Read the results

DNAble T16 Reader

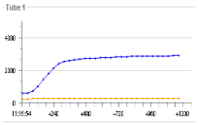
- Quickly place the ***clear resuspended*** **Master Mix** strip tubes into the DNAble Reader. Close lid of DNAble Reader and immediately **click "Start"** on software.

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

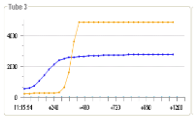
- After 15 minute assay time, the software will display results as negative (-), positive (+), or invalid (?).

  ***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 if the internal control curve varied from expected (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*

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

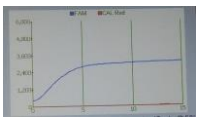
AmpliFire Reader

- When the instrument is ready push "**Start**". Place resuspended, capped clear strip tube into the AmpliFire Reader and press "**Ok**".

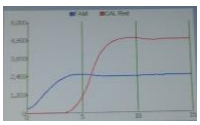
- After 15 minutes, the AmpliFire Reader will produce a short beeping sound and display final results. Results will be interpreted as Not Detected (-), Positive (+), or Invalid (!).

  Note: *The full assay time must be complete for result interpretation. 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 if the internal control curve varied from expected (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 AmpliFire Reader.



*Valid Negative*



*Valid Positive*

- After completion of the assay, carefully **remove run reaction strip tubes from instrument and place in opened foil pouch** (used to store Master Mix), seal and discard in waste container.

## LIMITED WARRANTY

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