DNAble® Sample Extraction Set 6

Catalog No. ACC-096 *Part #12138*

Set Contains:

- *MB9 Extraction Buffer*
- *MB1 Dilution Buffer*
- *1.5 mL clear micro-centrifuge tubes (50) for extraction*
- *1.5 mL blue micro-centrifuge tubes (50) for dilution*

Materials Not Provided:

- *Sterile Tryptic Soy Broth modified with 2% DNAble* Salmonella *Supplement (dTSB)*
- *Sterile collection sponge or swab for sampling surfaces Collection sponge should be pre-moistened with Sterile Dey-Engley broth.*
- *Precision pipette(s) capable of delivering 25-1000 µL*
- *Pipette tips*
- *Incubator capable of 37±1°C*
- *Dry heat block capable of 95±1°C, with insert suitable for 1.5 mL tubes*
- *Vortexer*
- *Micro-centrifuge capable of 10,000 x* g
- *Timer*
- *Polypropylene bags for culture*

Intended Use

When used with DNAble Kit for *Salmonella* Plus (DF-126) or the DNAble Kit for *Salmonella* Enteritidis (DF-056): This Set provides for extraction and detection of *Salmonella* DNA from a variety of matrices including liquid egg homogenate and shell eggs. DNAble *Salmonella* Supplement (Cat. No. XSALMD550) may be required for some matrices. Contact Technical Support for specific recommendations on other matrix types.

Intended User

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

How the Kit Works

An aliquot of MB9 buffer is added to a micro-centrifuge tube followed by a sample of mBPW-enriched culture. The sample is concentrated by centrifugation and heated to enable lysis of *Salmonella* cells. 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 culture
  - 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
- 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
- mBPW should be equilibrated at 37°C before use
- Filter bags should be used during enrichment to minimize particulates
- Enriched cultures should be mixed before sampling
- MB3 is stable for 1 year post manufacture when stored refrigerated at 4-8°C.
- **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" (link can be found here: www.envirologix.com/useful-links).

Enrichment

Notes

- Sample commodities to be tested according to your facility's sampling plan.
- Composite samples according to procedures defined for your facility.
- Where required, prepare 2% supplemented media using DNAble *Salmonella* Supplement (Cat. No. XSALMD550).
  - Prepare media according to manufacturer's instructions and allow cooling to 30°C before adding supplement.
  - Using aseptic technique, add DNAble *Salmonella* Supplement to media at a ratio of 2 to 100 and mix.
  - Once prepared, supplemented media may be stored refrigerated at 4-8°C for up to 9 months, protected from light.

Choose protocol based on sample type. Refer to the key listed with each sample type for applicability (**S+** = *Salmonella* Plus DF-126, **SE** = *Salmonella* Enteritidis DF-056):

Liquid egg homogenate (S+)	**Shell eggs (SE)**
1. Prepare 2% DNAble Modified TSB (dTSB) by addition of *Salmonella* Supplement (XSALMD550) to sterile TSB. 2. Add 375 mL liquid egg homogenate homogenized egg and 750 mL of dTSB to a culture bag. 3. Mix well by shaking, stomaching, or blending until the two liquids are well mixed. This step is crucial for optimal performance of the assay. 4. Incubate 20-22 hours at 37±1°C.	1. Prepare 2% DNAble Modified TSB (dTSB) by addition of *Salmonella* Supplement (XSALMD550) to sterile TSB. 2. Disinfect and sample 20 eggs, and homogenize as described in FDA-BAM (www.envirologix.com/useful-links) 3. Transfer to culture bag. 4. Add 2L of dTSB and mix well. 5. Incubate 20-22 hours at 37±1°C.

Contact Technical Service for additional matrix information and application guides for other matrices not listed herein

Sample Preparation

1. Pre-heat a dry heat block to 95°C. Verify heat block is holding temperature with ±1.5° using a simple thermometer.
2. Mix the culture before sampling. Transfer 1 mL of culture to a clear 1.5 mL micro-centrifuge tube supplied with the set.
3. Centrifuge the tube at 10,000 x *g* for 5 minutes (±30 seconds).
4. Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
5. **IMPORTANT: Shake the MB9 buffer containing bottle well before use**. It is important to mix the MB9 buffer immediately before pipetting.
6. Add 100 µL of MB9 buffer and vortex to suspend the pellet.
7. Heat the sample in the heat block at 95±1.5°C for 5±1 minutes.
8. Centrifuge the tube at 10,000 x *g* for 5 minutes (±30 seconds).
9. Shake the MB1 buffer containing bottle before use.
10. Place 90 µL of MB1 into a blue 1.5 mL micro-centrifuge tube using a fresh pipette tip.
11. Transfer 10 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
12. 5 µL of the sample from step 10 will be used in the subsequent DNAble reaction.

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