DNAble® Sample Extraction Set 3

Catalog No. ACC-089 *Part #11991*

Set Contains:

- *MB8 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 Modified Buffered Peptone Water (mBPW), 2% (*Salmonella *assays)*
- *Oxoid Novel Enrichment (ONE) broth (*Listeria *assay)*
- *Precision pipette(s) capable of delivering 20-1000 µL*
- *Pipette tips*
- *Incubator capable of 37±1°C*
- *Dry heat block capable of 95 and 100±1°C, with insert suitable for 1.5 mL tubes*
- *Vortexer*
- *Micro-centrifuge capable of 12,000 x* g
- *Timer*
- *Filter bags for culture*

Intended Use

- When used with DNAble Kits for ***Salmonella*** (**DF-026** and **DF-126**): This Set provides for extraction and detection of *Salmonella* DNA from a variety of matrices including the whole shrimp matrix. DNAble *Salmonella* Supplement (Cat. No. XSALMD550) may be required for some matrices. Contact Technical Support for specific recommendations on other matrix types.
- When used with DNAble Kit for ***Listeria monocytogenes*** (**DF-019**): This Set provides for extraction and detection of *Listeria* DNA from a variety of matrices including cheese and stainless steel surface swabs. Oxoid Novel Enrichment (ONE) broth may be required for some matrices. Contact Technical Support for specific recommendations on other matrix types.

Intended User

DNAble assays are 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 assays will be provided by EnviroLogix; contact Technical Service or visit envirologix.com for more information.

How the Kit Works

An aliquot of MB8 buffer is added to a micro-centrifuge tube followed by a sample of mBPW-enriched (*Salmonella*) or ONE broth-enriched (*Listeria*) culture. The sample is concentrated by centrifugation and heated to enable lysis of 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 and/or ONE broth 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
- MB8 is stable for 1 year post manufacture when stored refrigerated at 4-8°C.
- **Safety:** *Salmonella* and *Listeria* are pathogenic and are classified as a Biosafety Level 2 organisms. 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.

Enrichment

Notes

- Collect sample according to your facility's sampling plan.
- Composite samples according to procedures defined for your facility.
- For *Salmonella* assay(s): Prepare 2% mBPW using DNAble *Salmonella* Media Supplement.
  - Prepare BPW according to manufacturer's instructions and allow cooling to 30°C before adding supplement.
  - Using aseptic technique, add DNAble *Salmonella* supplement to BPW at a ratio of 2 to 100 and mix.
  - Once prepared, mBPW may be stored refrigerated at 4-8°C for up to three months, protected from light.
- For *Listeria* assay: ONE broth as per manufacturer's instructions.

Choose protocol based on sample type. Refer to the key listed with each sample type for applicability:
(S=*Salmonella* DF-026, S+=*Salmonella* Plus, Lm=*Listeria monocytogenes* DF-019)

Whole Shrimp (S, S+)	**Cheese (Lm)**	**Stainless Steel surface (Lm)**
1. Suspend 25 g of macerated shrimp per 225 mL of sterile mBPW. 2. Hand stomach or otherwise homogenize the sample for 2 minutes (±30 seconds). 3. Incubate 20-22 hours at 37±1°C.	4. Suspend 25 g samples in 225 mL sterile ONE broth 5. Use of filter culture bags is recommended. 6. Stomach or otherwise homogenize the sample for 2 minutes. 7. Incubate overnight 16-22 hours at 37°C.	1. Pre-moisten collection sponge with ONE broth. 2. Swab the surface using horizontal and vertical motions. 3. Place the sponge or swab in 225 mL of ONE broth. 4. Stomach by hand for 2 minutes. 5. Incubate suspended samples overnight 16-22 hours at 37°C.

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

Sample Preparation

***Salmonella* assays**	***Listeria* assay**
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. 3. Transfer 1 mL of culture to a clear 1.5 mL micro-centrifuge tube supplied with the set. 4. Centrifuge the tube at 10,000 x *g* for 5 minutes (±30 seconds). 5. Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed. 6. Add 100 µL of MB8 buffer to the pellet and briefly vortex to suspend the pellet. *IMPORTANT: Shake the MB8 vial before pipetting. This is to ensure that the undissolved particulate matter is homogenously distributed in the buffer.*	1. Pre-heat a dry heat block to 100°C. Verify heat block is holding temperature with ±1.5° using a simple thermometer. 2. Mix the culture before sampling. 3. Transfer 1 mL of enriched sample to a clear 1.5 mL micro-centrifuge tube. 4. Centrifuge the tube at 12,000 x *g* for 3 minutes (±30 seconds). 5. Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 10 µL) if an obvious pellet is not observed. 6. Re-suspend pellet in 1 mL sterile H2O 7. Centrifuge the tube at 12,000 x *g* for 1 minute. 8. Discard supernatant. 9. Add 100 µL of MB8 buffer to the pellet and briefly vortex to suspend the pellet.

7. Heat sample in heat block at 95±1.5°C for 5 minutes.
8. Centrifuge the tube at 10,000 x *g* for 5 minutes (±30 seconds).
9. Place 90 µL of MB1 into a blue 1.5 mL micro-centrifuge tube using a fresh pipette tip.
10. Transfer 10 µL of the supernatant from the second centrifugation into the MB1 and mix gently.
11. 5 µL of the sample from step 10 will be used in the subsequent DNAble reaction.

IMPORTANT: Shake MB8 bottle for 5 seconds before use. This is to ensure that the undissolved particulate matter is homogenously distributed in the buffer.

10. Heat the sample in the heat block at 100±1.5°C for 10 minutes.
11. Vortex briefly (~5 seconds) and centrifuge the tube at 12,000 x *g* for 1 minute.
12. Add 20 µL of the supernatant to 80 µL of MB1 using the blue dilution tubes.
13. 5 µL of the diluted sample will be used in the DNAble reaction.

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