DNAble® Sample Extraction Set 2

Set Contains:

- *MB3 Extraction 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%*
- *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*
- *Sterile Dey Engley (DE) culture broth (stainless steel surface testing only)*
- *Filter bags for culture*

Catalog No. ACC-085 *Part #11718*

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 dried pet food, wet pet food, stainless steel surfaces, poultry environmental swabs chicken carcass rinses, ground beef, powdered nonfat dry milk, lettuce, chocolate, and ground coriander. DNAble *Salmonella* Supplement (Cat. No. XSALMD550) may be required for some matrices. Contact Technical Support for specific recommendations on other matrix types. This product is certified as a *Performance Tested Method*℠ #041404 by the AOAC Research Institute as part of the DNAble *Salmonella* assay for use for dry pet food, stainless steel surfaces and poultry environmental drag swabs.

- When used with DNAble Kit for ***Salmonella*** **Enteritidis** (**DF-056**): This Set provides for extraction and detection of *Salmonella* Enteritidis DNA from a variety of matrices including boot swabs. DNAble *Salmonella* Supplement (Cat. No. XSALMD500) 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. They are designed for use by personnel with appropriate training in handling human pathogens, and in Microbiology and Molecular Assay techniques. Training specific to DNAble assays will be provided by EnviroLogix; contact Technical Service or visit envirologix.com/salmonella for more information.

How the Kit Works

An aliquot of MB3 buffer is added to a micro-centrifuge tube followed by a sample of 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* and *Salmonella* Enteritidis are pathogenic and classified as 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" (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 (XSALMD500)
  - 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* DF-026, **S+** = *Salmonella* Plus DF-126, **SE** = *Salmonella* Enteritidis DF-056):

Dry pet food (S, S+) 1. Suspend 25 g samples in 225 mL sterile mBPW. 2. Suspend 375 g samples in either 1125 or 3375 mL mBPW. Use of filter culture bags is recommended. 3. Allow the dry pet food to soften for 2-3 minutes, then stomach or otherwise homogenize the sample for 2 minutes (±30 seconds). 4. Incubate 20-22 hours at 37±1°C.	**Wet pet food (S+)** 1. Suspend 375 g samples in 1125 mL mBPW. Use of filter culture bags is recommended. 2. Allow the dry pet food to soften for 2-3 minutes, then stomach or otherwise homogenize the sample for 2 minutes (±30 seconds). 3. Incubate 20-22 hours at 37±1°C.	**Poultry environmental drag swabs (S, S+, SE)** 1. Place Swab in 100 mL of mBPW. 2. Shake the bag vigorously in an up-and-down motion at least ten times in an arc of approximately 30 cm over approximately 30 seconds 3. Incubate 20-22 hours at 37±1°C.
Stainless steel surface (S, S+) 1. Pre-moisten collection sponge or swab with sterile DE broth. 2. Swab the surface using horizontal and vertical motions. 3. Place the sponge or swab in 75 mL of mBPW. 4. Stomach by hand for 2 minutes (±30 seconds). 5. Incubate suspended samples 20-22 hours at 37±1°C.	**Lettuce (S+)** 1. Suspend 375 g samples in 1125 mL mBPW. Use of filter culture bags is recommended. 2. Stomach or otherwise homogenize the sample for 2 minutes (±30 seconds). 3. Incubate 20-22 hours at 37±1°C.	**Chicken carcass (S, S+)** 1. Rinse whole bird as instructed in USDA-MLG (www.envirologix.com/useful-links) 2. Use 30 ±0.5 mL of the sample rinse fluid obtained above for *Salmonella* analysis. Add 30 ±0.5 mL of sterile mBPW and mix well. 3. Incubate 20-22 hours at 37±1°C.

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Ground beef (S, S+)

1. Suspend 375 g samples in 1125 modified tryptone soy broth, mTSB+n (www.envirologix.com/useful-links). Use of filter culture bags is recommended. Do not confuse mTSB+n media (MLG) with DNAble Modified TSB (dTSB) prepared using EnviroLogix *Salmonella* Supplement (XSALMD550).
2. Stomach or otherwise homogenize the sample for 2 minutes (±30 seconds).
3. 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. If testing dry pet food, squeeze the culture (375 g with 1125 mL of mBPW) to aid in 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 MB3 buffer and vortex to suspend the pellet.
7. Heat the sample in the heat block at 95±1.5°C for 10±2 minutes.
8. Centrifuge the tube at 10,000 x *g* for 5 minutes (±30 seconds).

Choose next steps depending upon DNAble Assay:

S - *Salmonella* - DF-026

9. Place **100 µL** of MB3 into a blue 1.5 mL micro-centrifuge tube using a fresh pipette tip.
10. Transfer **25 µL** of the supernatant from the second centrifugation into the MB3 and mix gently.

S+ - *Salmonella* Plus - DF-126 and
SE - *Salmonella* Enteritidis - DF-056

9. Place **90 µL** of MB3 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 MB3 and mix gently.

11. 5 µL of the sample from step 10 will be used in the subsequent DNAble reaction.

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For Technical Support
Contact Us At:

EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103-1486 USA
Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail: dnable@envirologix.com

Website: **www.envirologix.com**

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