

PRODUCTS AVAILABLE

- 9807 **Reveal 2.0 one-step *Listeria* complete system for foods** – contains 20 devices, LESS medium and transfer pipettes for 20 tests
- 9806 **Reveal 2.0 for *Listeria* one-step complete system for environmental samples** – contains devices, LESS medium, environmental sampling kits and transfer pipettes for 20 tests
- 9808 **Reveal 2.0 for *Listeria* 48-hour enrichment complete system** – contains devices, Half Fraser Broth Plus medium, BLEB medium and transfer pipettes for 20 tests
- 9798 **LESS medium, unitized for foods** – contains 20 bottles of LESS medium for 20 food tests (225 mL)
- 9465 **Premoistened sampling sponges** – 100
- 9467 **Environmental sampling kit** – contains 20 cellulose sponges premoistened with neutralizing buffer, 20 pair of sterile plastic disposable gloves, and 20 twirl tie sample bags
- 9790A **Bulk LESS medium** – 500 g
- 9735 **Incubator with thermometer**
- 9275 ***Listeria* starter kit** – contains incubator, heating block, dry block base, 1000 culture tubes, pipette pump (10 mL), 500 sterile pipettes, 500 sterile bulb droppers
- 9276 **Pipettor** – 40–200 µL
- 9277 **Pipette pump** – 10 mL
- 9410 **Pipette tips** – 1000/bag
- 9411 **Heating block**
- 9412 **Dry block base**
- 9415 **Serological pipettes** – 10 mL, sterile (500)
- 9426 **Timer** (3 channel)
- 9427 **Electronic digital scale**
- 9700 **Neogen bulb droppers** – pack of 500)
- 9782 **Reveal for Half Fraser Broth Plus, unitized** – contains 20 bottles of Half Fraser Broth Plus for 20 samples
- 9785 **Reveal for Half Fraser Broth Plus** – 500 g
- 9783 **Reveal BLEB, unitized** – contains 20 bottles of BLEB for 20 tests
- 9786 **Reveal BLEB** – 500 g
- 9438 **Test tubes** – 13 x 100 mm glass test tube case of 1000
- 9792E **LESS medium, unitized** – contains 20 bottles of LESS medium for 20 environmental tests (100 mL)



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Read instructions carefully before starting test

Reveal 2.0 for *Listeria*

Store Reveal devices between 15–30°C when not in use.



INTENDED USE

The Reveal 2.0 for *Listeria* test system provides for rapid recovery of *Listeria* species in foods and environmental samples, allowing detection and presumptive identification of the test organism in 27–30 hours.

In an AOAC Research Institute Performance Tested MethodSM study, the Reveal 2.0 for *Listeria* test system utilizing LESS broth enrichment was found to be an effective procedure for detection of *Listeria* spp. in the following sample types: pasteurized crab meat, smoked salmon, Parmesan cheese, pasteurized liquid egg, ice cream, 2% milk, deli turkey, hot dogs, frozen cooked hamburgers, pepperoni, and sponge or swab samples from ceramic tile, plastic, stainless steel, and sealed concrete surfaces.

Results of inclusivity testing showed the Reveal 2.0 test devices detect all *Listeria* spp. except *L. grayi*.

ASSAY PRINCIPLES

This system utilizes *Listeria* enrichment single-step (LESS) medium to selectively enrich *Listeria* species present in food and environmental samples.

This system also uses Half Fraser Broth Plus and buffered *Listeria* enrichment broth (BLEB) to selectively enrich for *Listeria* species in select food matrices.

A sample (200 µL) equilibrated to room temperature after heat-kill of the enrichment culture is placed into the sample cup. The test device is placed into the sample and allowed to develop at ambient temperature for 20 minutes. The sample is wicked through a reagent zone which contains specific anti-*Listeria* antibodies conjugated to colloidal gold particles. If antigens are present in the sample, they will bind to the gold-conjugated antibodies. This antigen-antibody complex then leaves the reagent zone and travels through the nitrocellulose membrane which contains a zone of anti-*Listeria* antibodies. The immune complex with gold conjugate is captured and aggregates in this zone, thus displaying a visible line. The remainder of the sample continues to migrate to the end of the membrane where it is deposited into a waste reservoir.

The reagent zone also contains gold conjugate of a proprietary antigen which is eluted by the sample solution regardless of the presence of *Listeria* antigen. The gold-conjugated control indicator migrates through the membrane to the control capture zone (antibody to the proprietary antigen), where it is captured and aggregated to form a visible line. In the presence or absence of the *Listeria* antigen, the control line will form in the control zone, ensuring the test is working properly.

INTENDED USER

This test is designed for use by personnel familiar with the appropriate aseptic techniques for the isolation and identification of *Listeria* species. Training, which is available through Neogen, is recommended for those without a basic knowledge of microbiology.

MATERIALS PROVIDED

Reveal test kit (Neogen item 9707)

1. 20 Reveal for *Listeria* test devices
2. 20 transfer pipettes
3. 20 reaction cups

MEDIA REQUIRED BUT NOT PROVIDED (BASED ON SAMPLE TYPE)

• Reveal enrichment for environmental sponges (Neogen item 9792E)

1. 20 bottles of LESS media unitized for environmental samples

• Reveal single step enrichment for foods (Neogen item 9798)

1. 20 bottles of LESS media unitized for foods

• Reveal 48-hour enrichment (Neogen item 9780)

1. 20 bottles of Half Fraser Broth Plus (Neogen item 9782)
2. 20 bottles of BLEB (Neogen item 9785)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Incubator at 30 ± 1°C (Neogen item 9735)
2. Water bath or heater block at 80°C (Neogen items 9411, 9412)
3. 13 x 100 mm glass test tubes (Neogen item 9438)
4. Pipettor capable of delivering 200 µL (Neogen item 9276) or transfer pipettes
5. Sterile purified water

STORAGE

Store *Listeria* test devices at 15–30°C when not in use. Store environmental sampling kit and dry media below 30°C.

PRECAUTIONS

1. Do not autoclave LESS medium.
2. Do not use test devices or culture media beyond the expiration date.
3. Use rehydrated media the same day as rehydration.
4. Incubation times other than those specified may lead to erroneous results.
5. Sample bags must be closed loosely to allow air exchange during incubation, which is vital for growth promotion.
6. Use good microbiological laboratory practices.

SAMPLE PREPARATION AND ENRICHMENT

Food samples with LESS medium

1. Transfer the contents of 1 bottle of LESS medium unitized for foods (Neogen item 9798) or 17.6 g of bulk LESS medium (Neogen item 9790A) into a Stomacher-type bag. Using the graduated cup provided, add 225 mL of sterile water. Grasp the bag tightly 2–3 inches from the top, and mix vigorously until dissolved.
2. Place 25 g of the sample into the Stomacher-type bag and homogenize by placing in a Stomacher for **30 seconds**.
3. Incubate at 30 ± 1°C for **27–30 hours**.

Food samples with Half Fraser Broth Plus and BLEB

NOTE: Not part of AOAC Performance Tested Method.

1. Transfer the contents of 1 bottle of unitized Half Fraser Broth Plus (Neogen item 9782) or 12.4 g bulk Half Fraser Broth Plus (Neogen item 9785) into a Stomacher-type bag. Using the graduated cup provided, add 225 mL of sterile water. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Place 25 g of the sample into the Stomacher-type bag. Grasp the bag tightly at the top and knead sample until mixed. **ALTERNATIVE:** Place bag in a Stomacher apparatus and mix for **30 seconds** at normal speed.
3. Incubate the loosely closed bag at 30 ± 1°C for **21–24 hours**.
4. Prior to removing sample from incubator, rehydrate 1 bottle of BLEB by adding 10 mL of sterile water. Cap tightly and shake to dissolve medium.
5. Remove Half Fraser Broth sample from incubator, mix well and transfer 0.1 mL (3 free falling drops) into the bottle of rehydrated BLEB.
6. Incubate the loosely capped bottle of BLEB at 30 ± 1°C for an additional **21–24 hours**.

Sponge samples with LESS medium

1. Prepare enough LESS medium to achieve an approximate 10-to-1 broth-to-sponge ratio (based on the hydrated volume of the sponge). For typical sampling sponges of approximately 80 x 40 x 4 mm, enrichment broth volume of 100 mL is recommended. Transfer the contents of 1 bottle unitized LESS medium for environmental samples (Neogen item 9792E) or 7.8 g of LESS medium (Neogen item 9790A) into a Stomacher-type bag and add 100 mL of sterile water using the graduated cup provided. Grasp the bag tightly 2–3 inches from the top, and mix vigorously until dissolved.
2. Place the sample sponge into the Stomacher-type bag. Grasp the bag tightly at the top and shake vigorously using a side-to-side motion. **ALTERNATIVE:** Place bag in Stomacher apparatus and mix for **30 seconds** at normal speed.
3. Incubate for **27–30 hours** at 30 ± 1°C.

Sponge samples with Half Fraser Broth Plus and BLEB

NOTE: Not part of AOAC Performance Tested Method.

1. Prepare enough Half Fraser Broth Plus to achieve an approximate 10-to-1 broth-to-sponge ratio (based on the hydrated volume of the sponge). For typical sampling sponges of approximately 80 x 40 x 4 mm, enrichment broth volume of 100 mL is recommended. To rehydrate this volume, transfer 5.5 g of bulk Half Fraser Broth Plus (Neogen item 9785) into a Stomacher-type bag and add 100 mL of sterile water using the graduated cup provided. Grasp the bag tightly 2–3 inches from the top, and mix vigorously until dissolved.
2. Place the sample sponge into the Stomacher-type bag. Grasp the bag tightly at the top and shake vigorously using a side-to-side motion. **ALTERNATIVE:** Place bag into Stomacher apparatus and mix for **30 seconds** at normal speed.
3. Incubate the loosely closed bag at 30°C for a minimum of **21 hours**. Do not exceed 24 hours.

4. Prior to removing sample from the incubator, rehydrate 1 bottle of BLEB by adding 10 mL of sterile water. Cap tightly and shake to dissolve medium.
5. Remove Half Fraser Broth sample from incubator. Mix well and transfer 0.1 mL (3 free falling drops) into the bottle of rehydrated BLEB.
6. Incubate the loosely capped bottle of BLEB at 30 ± 1°C for an additional **21–24 hours**.

Swab samples with LESS medium

1. Transfer the contents of 1 bottle of LESS medium unitized for environmental samples (Neogen item 9792E) or 7.8 g of bulk LESS medium (Neogen item 9790A) into a Stomacher-type bag. Using the graduated cup provided, add 100 mL of sterile water. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Transfer 10 mL prepared LESS medium into a clean sterile test tube.
3. Break off the swab sample into the test tube. Cap tightly and shake by inverting the test tube several times. **ALTERNATIVE:** Mix the capped test tube by vortex.
4. Incubate for **27–30 hours** at 30 ± 1°C.

Swab samples with Half Fraser Broth Plus and BLEB

NOTE: Not part of AOAC Performance Tested Method.

1. Transfer the contents of 1 bottle of unitized Half Fraser Broth Plus (Neogen item 9782) or 5.5 g of bulk Half Fraser Broth Plus (Neogen item 9785) into a Stomacher-type bag. Using the graduated cup provided, add 100 mL of sterile water. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Transfer 10 mL prepared Half Fraser Broth Plus into a clean sterile test tube.
3. Break off the swab sample into the test tube. Cap tightly and shake by inverting the test tube several times. **ALTERNATIVE:** Mix the capped test tube by vortex.
4. Incubate the loosely capped test tube at 30°C for a **minimum of 21 hours**. Do not exceed 24 hours.
5. Prior to removing the sample from the incubator, rehydrate 1 bottle of BLEB by adding 10 mL of sterile water. Cap tightly and shake to dissolve medium.
6. Remove Half Fraser Broth sample from incubator. Mix well and transfer 0.1 mL (3 free falling drops) into the bottle of rehydrated BLEB.
7. Incubate the loosely capped bottle of BLEB at 30 ± 1°C for an additional **21–24 hours**.

FINAL SAMPLE PREPARATION

1. Carefully remove the sample from the incubator. Mix sample well and transfer about 2 mL of the enriched sample to a small glass test tube.
2. Place the glass tube in a water bath or heater block at 80°C for **20 minutes**.
3. Cool to room temperature.

REVEAL TEST PROTOCOL

1. Remove the required number of Reveal 2.0 for *Listeria* test devices from container.
2. Transfer 200 µL or 8 drops of heat-killed enriched sample to the Reveal sample cup.
3. Place Reveal device into sample cup containing sample and incubate at ambient temperature for **20 minutes**.
4. Record Reveal results after **20 minutes**.

INTERPRETATION OF RESULTS

Visual interpretation

1. Line in both control and test zone in 20 minutes is considered positive.
2. Line in control only at 20 minutes is considered negative.
3. If no line appears in the control zone, the test is considered invalid and another device should be tested.

Optional electronic interpretation

1. Place the Reveal test device into the Reveal AccuScan® III Reader according to the instrument instructions and follow the onscreen prompts to interpret and record the device's test result.

NOTE: The Reveal device forms a distinct line in the test region when *Listeria* are present; the intensity of the line may be variable based on serovar and/or concentration. If a distinct visible line forms, regardless of intensity, the sample should be considered positive.

CONFIRMATION

Neogen recommends that presumptively positive Reveal enrichment culture be verified by plating the sample culture onto media described in USDA-MLG or FDA-BAM, contingent on the sample type.

NOTE: Testing separate samples via alternate methods may not yield the same result. Bacteria are not evenly distributed within a lot of material hence the additional sample may not contain the target organism.

DISPOSAL

Dispose (autoclave, bleach, etc.) of Reveal test devices, pipettes, and media in accordance with all applicable local, state and federal regulations.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Service can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's Food Safety test kits, on Neogen's website at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

WARRANTY

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