

Actero™ Listeria Enrichment Media Product Information

INTENDED USE:

Actero™ Listeria Enrichment Media (“Actero™ Listeria”) is a selective medium, specifically optimized for single-step recovery and enrichment of *Listeria* spp. from food and environmental samples.

PRINCIPLE OF OPERATION:

Actero™ Listeria Enrichment Media, “Actero™ Listeria”, combines nutritional components with additional ingredients that are necessary to selectively improve the recovery and growth of *Listeria*. The selective agents present in the Actero™ Listeria have been optimized to efficiently target competing normal bacterial flora without affecting the growth of *Listeria* spp.

CONTENTS:

Dehydrated Actero™ Listeria Enrichment Media is provided in **500 g** (Cat# FCM-011, BBFCM-011), **2 kg** (Cat# FCM-022, BBFCM-022), **5 kg** (Cat. # FCM-199), and **10 kg** (Cat# FCM-023).

ADDITIONAL MATERIALS REQUIRED:

All Samples

1. Distilled/deionized water.
2. Sterile Stomacher® bags or equivalent with and without a filter.
3. Stomacher® or equivalent.
4. Calibrated 10 µL plastic inoculating loops.
5. Rapid™L mono agar plates (RLM).
6. Horse blood overlay agar (HL).
7. Modified Oxford agar plates (MOX).
8. Incubator: at 29 ± 0.5°C, 32 ± 0.5°C and 35 ± 2°C.
9. Regular laboratory equipment is also required.

Environmental Samples

1. Non-bactericidal sterile cellulose sampling sponges (8×4×0.3 cm) pre-moistened with neutralizing Dey-Engley buffer (D/E) (Salus Scientific Cat # FCLS-005).

PROCEDURE:

Actero™ Listeria Enrichment Media Preparation

Autoclave Method

1. Prepare a clean bottle for each liter of medium preparation.
2. Shake the container of dry enrichment medium before each use.
3. Measure 53.8 g of powder into the bottle and add to 1 L of distilled water.
4. Constantly stir and heat the solution until the powder is dissolved (avoid boiling). The pH should be at 7.2 ± 0.2.
5. Sterilize the bottle of prepared medium by autoclaving at 110°C for 15 min.
6. Cool the bottle to room temperature and store it at 2–8°C for up to 45 days. Keep away from light.

Non-Autoclave Method

1. Prepare a sterile and clean bottle for each liter of medium preparation.
2. Shake the container of dry enrichment medium before each use.
3. Measure 53.8 g of powder into the bottle and add to 1 L of **sterile** distilled water.
4. Constantly stir and heat the solution until the powder is dissolved (avoid boiling). The pH should be at 7.2 ± 0.2.
5. Cool the prepared medium to the appropriate temperature (29, 32 °C or 35°C) and use immediately.

Note: improper preparation may result in floating particles or turbidity in the medium after autoclaving.

Environmental Surface Sample Preparation

1. Use a non-bactericidal sterile cellulose sampling sponge (8×4×0.3 cm) that has been pre-moistened with D/E buffer.
2. Swab the surface to be tested with one side of the swab(s) in a horizontal direction (approximately 10 cm), and with the other side in a vertical direction (approximately 10 cm) back and forth (one stroke back and one stroke forward) to swab the entire area of 100 cm².
3. Place each surface sampled sponge in a sterile sample bag, and keep it at 4 ± 2°C until it is ready for testing. Analyze sample units as soon as possible after their reception in the laboratory.
4. When ready to test, pre-warm the prepared Actero™ Listeria at **29, 32 or 35°C** depending on the detection method to be used.
5. Add **90 mL** of the pre-warmed Actero™ Listeria to each sponge sample in its sample bag.
6. Homogenize the sample for **30 seconds** in a Stomacher® 400 circulator or equivalent. Hand mixing is an acceptable alternative for stomaching. To hand mix, massage each sponge that is in the sealed bag for approximately one minute.

Enrichment of Environmental Surface Samples Using Actero™ Listeria Enrichment Media

Actero™ Listeria Culture Method

Food Contact Surface Samples (Stainless Steel, Plastic)

Close the bags and incubate the samples in an incubator for **24 h at 29 ± 0.5°C** or for **28 h at 32 ± 0.5°C**.

Non-Food Contact Surface Samples (Ceramic, Sealed Concrete, Rubber)

Close the bags and incubate the samples in an incubator for **28 h at 32 ± 0.5°C**.

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ Listeria Culture Method

Environmental Surface Samples (Stainless Steel, Plastic, Sealed Concrete)

Close the bags and incubate the samples in an incubator for **20-24 h at 35 ± 2°C**.

1. *Note: Adherence to temperature is important for accurate results.*

Enrichment of Samples Using Actero™ Listeria Enrichment Media

NOTE: The sample preparation depends on the type and size of the sample. Thus, the protocol to prepare the sample should be chosen according to those conditions. However, all the other steps are the same.

Food Sample Preparation

Liquid Whole Eggs (25 g)

Actero™ Listeria Culture Method

1. Add **150 mL** of the pre-warmed (**32°C**) Actero™ Listeria Enrichment Media to each **25 g** sample in a **non-filtered** Stomacher® sample bag or equivalent.
2. Homogenize the sample for **30 seconds at 90 rpm** in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for 28 h at 32 ± 0.5°C. Adherence to temperature is important for accurate results.

Summary of Enrichment and Analysis of Samples Using Actero™ Listeria Enrichment Media

Sample Type	Sample Preparation	Analysis of Enriched Samples
Environmental Sample		
Food Contact Surface ➤ Stainless steel ➤ Plastic	1. Swab a 100 cm ² surface with a sponge (pre-moistened with D/E buffer) and keep it in a sterile bag at 4°C until tested. 2. Homogenize sponge with 90 mL pre-warmed (29°C or 32°C) Actero™ Listeria Enrichment Media. 3. Incubate at 29 ± 0.5°C for 24 h or at 32 ± 0.5°C for 28 h.	✓ By Actero™ Listeria Culture Method
	1. Swab a 100 cm ² surface with a sponge (pre-moistened with D/E buffer) and keep it in a sterile bag at 4°C until tested. 2. Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 3. Incubate at 35 ± 2°C for 20-24 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
Non-Food Contact Surface ➤ Rubber ➤ Sealed concrete ➤ Ceramic	1. Swab a 100 cm ² surface with a sponge and keep it in a sterile bag at 4°C until tested. 2. Homogenize sponge with 90 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. 3. Incubate at 32 ± 0.5°C for 28 h.	✓ By Actero™ Listeria Culture Method
Non-Food Contact Surface ➤ Sealed concrete	1. Swab a 100 cm ² surface with a sponge and keep it in a sterile bag at 4°C until tested. 2. Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 3. Incubate at 35 ± 2°C for 20-24 h.	✓ By BAX® System Real-Time PCR Assays for genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
Food Sample		
➤ Liquid whole eggs	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. 2. Incubate at 32 ± 0.5°C for 28 h.	✓ By Actero™ Listeria Culture Method
➤ Cold smoked salmon	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (29°C) Actero™ Listeria Enrichment Media. 2. Incubate at 29 ± 0.5°C for 28 h.	✓ By Actero™ Listeria Culture Method
	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 22-24 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Frozen cooked shrimp	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. 2. Incubate at 32 ± 0.5°C for 28 h.	✓ By Actero™ Listeria Culture Method
	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 22-24 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Raw frozen salmon	1. Homogenize (30 s) 25 g sample with 100 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 18-22 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Soft fresh cheese (Ricotta)	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. 2. Incubate at 32 ± 0.5°C for 28 h.	✓ By Actero™ Listeria Culture Method
➤ Mexican-style cheese	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 22-24 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Ice cream	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 18-22 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method

Sample Type	Sample Preparation	Analysis of Enriched Samples
➤ Pasteurized milk	1. Homogenize (30 sec) 25 g sample with 100 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 0.5°C for 22-24 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
	1. Homogenize (30 sec) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. 2. Incubate at 32 ± 0.5°C for 28 h.	✓ By Actero™ Listeria Culture Method
➤ Bagged fresh spinach	1. Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 22-24 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Hot dog sausage	1. Homogenize (30 s) 125 g sample with 750 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 26-28 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Smoked turkey breast	1. Homogenize (30 s) 125 g sample with 500 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 24-28 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Cured ham	1. Homogenize (30 s) 125 g sample with 500 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. 2. Incubate at 35 ± 2°C for 26-28 h.	✓ By BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method

Cold Smoked Salmon (25 g)

Actero™ Listeria Culture Method

1. Add 150 mL of the pre-warmed (29°C) Actero™ Listeria Enrichment Media to each 25 g sample in a filtered Stomacher® sample bag or equivalent.
2. Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for 28 h at 29 ± 0.5°C. Adherence to temperature is important for accurate results.

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ Listeria Culture Method

1. Add 150 mL of the pre-warmed (35°C) Actero™ Listeria Enrichment Media to each 25 g cold smoked salmon sample in a filtered Stomacher® sample bag or equivalent.
2. Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for 22-24 h at 35 ± 2°C.

Frozen Cooked Shrimp (25 g)

Actero™ Listeria Culture Method

1. Add 150 mL of the pre-warmed (32°C) Actero™ Listeria Enrichment Media to each 25 g sample in a filtered Stomacher® sample bag or equivalent.
2. Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for 28 h at 32 ± 0.5°C. Adherence to temperature is important for accurate results.

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ Listeria Culture Method

1. Add 150 mL of the pre-warmed (35°C) Actero™ Listeria Enrichment Media to each 25 g sample in a filtered Stomacher® sample bag or equivalent.
2. Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent.

3. For the enrichment phase, close the bags and incubate the samples in an incubator for 22-24 h at 35 ± 2°C.

Raw Frozen Salmon (25 g)

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ Listeria Culture Method

1. Add 100 mL of the pre-warmed (35°C) Actero™ Listeria Enrichment Media to each 25 g raw frozen salmon sample in a filtered Stomacher® sample bag or equivalent.
2. Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for 18-22 h at 35 ± 2°C.

Soft Fresh Cheese Ricotta (25 g)

Actero™ Listeria Culture Method

1. Add 150 mL of the pre-warmed (32°C) Actero™ Listeria Enrichment Media to each 25 g sample in a filtered Stomacher® sample bag or equivalent.
2. Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for 28 h at 32 ± 0.5°C. Adherence to temperature is important for accurate results.

Mexican-style cheese (25 g), Bagged Fresh Spinach (25 g)

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ Listeria Culture Method

1. Add 150 mL of the pre-warmed (35°C) Actero™ Listeria Enrichment Media to each 25 g sample in a filtered Stomacher® sample bag or equivalent.
2. Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for 22-24 h at 35 ± 2°C.

Ice Cream (25 g)

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ *Listeria* Culture Method

1. Add **150 mL** of the pre-warmed (**35°C**) Actero™ *Listeria* Enrichment Media to each **25 g** sample in a **filtered** Stomacher® sample bag.
2. Homogenize the sample for **30 seconds** in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for **18-22 h at 35 ± 2°C**.

Pasteurized Milk (25 g)

Actero™ *Listeria* Culture Method

1. Add **150 mL** of the pre-warmed (**32°C**) Actero™ *Listeria* Enrichment Media to each **25 g** sample in a **non-filtered** Stomacher® sample bag or equivalent.
2. Homogenize the sample for **30 seconds** in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for **28 h at 32 ± 0.5°C**. Adherence to temperature is important for accurate results.

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ *Listeria* Culture Method

1. Add **100 mL** of the pre-warmed (**35°C**) Actero™ *Listeria* Enrichment Media to each **25 g** sample in a **filtered** Stomacher® sample bag or equivalent.
2. Homogenize the sample for **30 seconds** in a Stomacher® 400 circulator or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for **22-24 h at 35 ± 2°C**.

Hot Dog Sausage (125 g)

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ *Listeria* Culture Method

1. Add **750 mL** of the pre-warmed (**35°C**) Actero™ *Listeria* Enrichment Media to each **125 g** sample in a **filtered** Stomacher® sample bag or equivalent.
2. Homogenize the sample for **30 seconds** in a Stomacher® 3500 or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for **26-28 h at 35 ± 2°C**. Adherence to temperature is important for accurate results.

Smoked Turkey Breast (125 g)

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ *Listeria* Culture Method

1. Add **500 mL** of the pre-warmed (**35°C**) Actero™ *Listeria* Enrichment Media to each **125 g** sample in a **filtered** Stomacher® sample bag or equivalent.
2. Homogenize the sample for **30 seconds** in a Stomacher® 3500 or equivalent.
3. For the enrichment phase, close the bags and incubate the samples in an incubator for **24-28 h at 35 ± 2°C**. Adherence to temperature is important for accurate results.

Cured Ham (125 g)

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* or Actero™ *Listeria* Culture Method

1. Add **500 mL** of the pre-warmed (**35°C**) Actero™ *Listeria* Enrichment Media to each **125 g** sample in a **filtered** Stomacher® sample bag or equivalent.
2. Homogenize the sample for **30 seconds** in a Stomacher® 3500 or equivalent.

3. For the enrichment phase, close the bags and incubate the samples in an incubator for **26-28 h at 35 ± 2°C**. Adherence to temperature is important for accurate results.

Analysis of Enriched Samples

Actero™ *Listeria* Culture Method – All samples

1. At the end of the enrichment phase, streak a loopful of an enriched sample over the surface of one MOX and one RLM agar plates. Incubate the streaked plates for 24-48 h at 35 ± 2°C in an incubator.
2. After the 24 h incubation, examine both the MOX and RLM agar plates for colonies with morphology typical for *Listeria* spp. with a following re-examination after 48 h of total incubation.
3. Use a loop to contact a minimum of 20 (if available) suspect colonies presented in MOX agar plate and collectively streak for isolation on one or more HL agar plates. Incubate the streaked HL plates at 35 ± 2°C for 22 ± 4 h.
4. Confirm the presumptive colonies growing on HL agar plates as *Listeria* spp. as recommended in the U.S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 10, Detection and Enumeration of *Listeria monocytogenes* (2022) or in the FSIS Microbiology Laboratory Guidebook Chapter 8.13 (2021).

Interpretation and Test Result Report

1. If **no suspect colonies** with morphology typical of *Listeria* spp. have been found on MOX (typical *Listeria* spp. are small (≈1 mm), black, round in shape and are surrounded by a darker zone) and/or RLM agar plates (*L. monocytogenes* colonies will appear blue (pale blue, grey-blue to dark blue) without a yellow halo, *L. ivanovii* colonies will appear blue-green with a yellow halo and other *Listeria* strains will be white, with or without a yellow halo) following a 48 ± 2 h of total incubation, the sample is considered as being **negative for the presence of *Listeria* spp.**
2. If the **suspect colonies** with morphology typical for *Listeria* spp. (see above) have been found on MOX and/or RLM agar plates after 48 h of total incubation, the sample should be considered as **potentially positive for *Listeria* spp.** The suspect colonies **must be confirmed** according to the U.S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 10, Detection and Enumeration of *Listeria monocytogenes* (2022) or in the FSIS Microbiology Laboratory Guidebook Chapter 8.13 (2021).

BAX® System Real-Time PCR Assays for Genus *Listeria* and for *Listeria monocytogenes* – Environmental surface samples

Refer to the Test Protocol section of the package insert for each respective assay.

PRODUCT STORAGE AND SHELF LIFE:

The dehydrated medium should be kept at 12–25°C, in a tightly closed bottle in a dry well-ventilated place protected from light. The expiry date is indicated on the package.

If the medium has been autoclaved, it can be stored at 2–8°C for up to 45 days, in a tightly closed bottle in a cool dry place protected from light. If the medium has not been autoclaved, it should be used immediately.

DISPOSAL:

Dispose of all materials used and the enrichment medium by autoclaving or according to approved practices.

Ensure that all biohazard waste is disposed of according to local, municipal, provincial, state and/or federal regulations.

PRECAUTIONS:

Biosafety level 2 procedures should be exercised.
(<https://www.cdc.gov/labs/BMBL.html>).

Extreme care should be taken in handling test samples and enrichment broths. All enrichment broths may contain various pathogens whether they contain *Listeria spp.* or not. Moreover, those who are at the highest risk of serious illness like pregnant women, and potentially immunocompromised individuals should have limited access to laboratory rooms or areas where *L. monocytogenes* isolation or identification procedures are in progress.

TERMS AND CONDITIONS:

Salus Scientific Inc. makes no representations and warranties concerning its products other than those stated herein. All Product(s) delivered hereunder by Salus Scientific Inc., its affiliates or any other person on its behalf shall, at the time of delivery, be manufactured to meet Salus Scientific Inc.'s specifications and all applicable laws. All other terms, conditions and

warranties, including any warranty of merchantability, quality, fitness or suitability for a particular or intended purpose, implied by common law or statute, (implied warranties) are expressly excluded.

CATALOGUE NUMBER:

FCM-011 (BBFCM-011): Actero™ *Listeria* Enrichment Media, 500 g
FCM-022 (BBFCM-022): Actero™ *Listeria* Enrichment Media, 2 kg
FCM-023: Actero™ *Listeria* Enrichment Media, 10 kg
FCM-199: Actero™ *Listeria* Enrichment Media, 5 kg

FOR FURTHER INFORMATION PLEASE CONTACT:

Salus Scientific Inc.
Suite 450, 1414 – 8 St. S.W.
Calgary, Alberta, Canada
T2R 1J6
Tel: 1-877-298-0208

