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 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 Bottle (Cat# FCM-011, BBFCM-011) or in 2 kg Bottle (Cat# FCM-022, BBFCM-022). Each format contains sufficient amount of medium powder to prepare respectively 9.29 L and 37.16 L of liquid medium.

ADDITIONAL MATERIALS REQUIRED:

All Samples
1. Distilled/deionized water.
2. Sterile stomacher® bags or equivalent with and without 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) 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 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** or **FoodChek™ Listeria spp. assay**

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.

**DuPont™ 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 24-24 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 in function of those conditions. However, all the other steps are the same.

**Food Sample Preparation**

**Liquid Whole Eggs (25 g)**

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sample Preparation</th>
<th>Analysis of Enriched Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environmental Sample</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Contact Surface</td>
<td>1. Swab a 100 cm² surface with a sponge (pre-moistened with D/E buffer) and keep it in a sterile bag at 4°C until tested.</td>
<td>By FoodChek™ Listeria spp. Assay</td>
</tr>
<tr>
<td></td>
<td>2. Homogenize sponge with <strong>90 mL pre-warmed (29°C or 32°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By Actero™ Listeria Culture Method</td>
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<tr>
<td></td>
<td>3. Incubate at <strong>29 ± 0.5°C for 24 h or at 32 ± 0.5°C for 28 h.</strong></td>
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</tr>
<tr>
<td>Non Food Contact Surface</td>
<td>1. Swab a 100 cm² surface with a sponge and keep it in a sterile bag at 4°C until tested.</td>
<td>By DuPont™ BAX® System Real-Time PCR Assays for Genus <em>Listeria</em> and <em>L. monocytogenes</em></td>
</tr>
<tr>
<td>Rubber</td>
<td>2. Homogenize sponge with <strong>90 mL pre-warmed (32°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
<tr>
<td>Sealed concrete</td>
<td>3. Incubate at <strong>32 ± 0.5°C for 28 h.</strong></td>
<td></td>
</tr>
<tr>
<td>Sealed concrete</td>
<td>1. Swab a 100 cm² surface with a sponge and keep it in a sterile bag at 4°C until tested.</td>
<td>By DuPont™ BAX® System Real-Time PCR Assays for Genus <em>Listeria</em> and <em>L. monocytogenes</em></td>
</tr>
<tr>
<td>Ceramic</td>
<td>2. Homogenize sponge with <strong>90 mL pre-warmed (35°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By Actero™ Listeria Culture Method</td>
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<tr>
<td></td>
<td>3. Incubate at <strong>35 ± 2°C for 20-24 h.</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Food Sample</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid whole eggs</td>
<td>1. Homogenize (<strong>30 s</strong> 25 g sample with <strong>150 mL pre-warmed (32°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By FoodChek™ Listeria spp. Assay</td>
</tr>
<tr>
<td></td>
<td>2. Incubate at <strong>32 ± 0.5°C for 28 h.</strong></td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
<tr>
<td>Cold smoked salmon</td>
<td>1. Homogenize (<strong>30 s</strong> 25 g sample with <strong>150 mL pre-warmed (29°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By FoodChek™ Listeria spp. Assay</td>
</tr>
<tr>
<td></td>
<td>2. Incubate at <strong>29 ± 0.5°C for 28 h.</strong></td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
<tr>
<td>Frozen cooked shrimp</td>
<td>1. Homogenize (<strong>30 s</strong> 25 g sample with <strong>150 mL pre-warmed (32°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By DuPont™ BAX® System Real-Time PCR Assays for Genus <em>Listeria</em> and <em>L. monocytogenes</em></td>
</tr>
<tr>
<td></td>
<td>2. Incubate at <strong>35 ± 2°C for 22-24 h.</strong></td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
<tr>
<td>Raw frozen salmon</td>
<td>1. Homogenize (<strong>30 s</strong> 25 g sample with <strong>100 mL pre-warmed (35°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By DuPont™ BAX® System Real-Time PCR Assays for Genus <em>Listeria</em> and <em>L. monocytogenes</em></td>
</tr>
<tr>
<td></td>
<td>2. Incubate at <strong>35 ± 2°C for 18-22 h.</strong></td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
<tr>
<td>Soft fresh cheese (Ricotta)</td>
<td>1. Homogenize (<strong>30 s</strong> 25 g sample with <strong>150 mL pre-warmed (32°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By FoodChek™ Listeria spp. Assay</td>
</tr>
<tr>
<td></td>
<td>2. Incubate at <strong>32 ± 0.5°C for 28 h.</strong></td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
<tr>
<td>Mexican-style cheese</td>
<td>1. Homogenize (<strong>30 s</strong> 25 g sample with <strong>150 mL pre-warmed (35°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By DuPont™ BAX® System Real-Time PCR Assays for Genus <em>Listeria</em> and <em>L. monocytogenes</em></td>
</tr>
<tr>
<td></td>
<td>2. Incubate at <strong>35 ± 2°C for 22-24 h.</strong></td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
<tr>
<td>Ice cream</td>
<td>1. Homogenize (<strong>30 s</strong> 25 g sample with <strong>150 mL pre-warmed (35°C)</strong> Actero™ Listeria Enrichment Media.</td>
<td>By DuPont™ BAX® System Real-Time PCR Assays for Genus <em>Listeria</em> and <em>L. monocytogenes</em></td>
</tr>
<tr>
<td></td>
<td>2. Incubate at <strong>35 ± 2°C for 18-22 h.</strong></td>
<td>By Actero™ Listeria Culture Method</td>
</tr>
</tbody>
</table>
### 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 DuPont™ BAX® System Real-Time PCR Assays for Genus Listeria and *L. monocytogenes*  
✓ By Actero™ Listeria Culture Method

| Pasturized milk | Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent. 2. 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. |
|---|---|---|

**Bagged fresh spinach** | 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 and for *L. monocytogenes* and for Genus *Listeria* and *L. monocytogenes* and for Actero™ Listeria Culture Method

| Bagged fresh spinach | Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent. 2. 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. |
|---|---|---|

**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 DuPont™ BAX® System Real-Time PCR Assays for Genus Listeria and *L. monocytogenes*  
✓ By Actero™ Listeria Culture Method

| Hot dog sausage | Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent. 2. For the enrichment phase, close the bags and incubate the samples in an incubator for 22-24 h at 35 ± 2°C. |
|---|---|---|

**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 DuPont™ BAX® System Real-Time PCR Assays for Genus Listeria and *L. monocytogenes*  
✓ By Actero™ Listeria Culture Method

| Smoked turkey breast | Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent. 2. For the enrichment phase, close the bags and incubate the samples in an incubator for 22-24 h at 35 ± 2°C. |
|---|---|---|

**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 DuPont™ BAX® System Real-Time PCR Assays for Genus Listeria and *L. monocytogenes*  
✓ By Actero™ Listeria Culture Method

| Cured ham | Homogenize the sample for 30 seconds in a Stomacher® 400 circulator or equivalent. 2. For the enrichment phase, close the bags and incubate the samples in an incubator for 22-24 h at 35 ± 2°C. |
|---|---|---|

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.

**Cold Smoked Salmon (25 g)**
*Actero™ Listeria Culture Method or FoodChek™ Listeria spp. assay*
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.

**Raw Frozen Salmon (25 g)**
*DuPont™ BAX® System Real-Time PCR Assays for Genus *Listeria* and for *L. 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 or FoodChek™ Listeria spp. assay*
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.

**Frozen Cooked Shrimps (25 g)**
*Actero™ Listeria Culture Method or FoodChek™ Listeria spp. assay*
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)**
*DuPont™ BAX® System Real-Time PCR Assays for Genus *Listeria* and for *L. 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)
DuPont™ 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.

Hot Dog Sausage (125 g)
DuPont™ 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)
DuPont™ 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)
DuPont™ 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 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.

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 (2011) or in the FSIS Microbiology Laboratory Guidebook Chapter 8.09 (2013).

FoodChex™ Listeria spp. Assay – All samples
Refer to the Analysis section of the package insert for the FoodChex™ Listeria spp. assay.

DuPont™ 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 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 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.
(BMBL, http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf). 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:
FoodChek Systems Inc. makes no representations and warranties concerning its products other than those stated herein. All Product(s) delivered hereunder by FoodChek Systems Inc., its affiliates or any other person on its behalf shall, at the time of delivery, be manufactured to meet FoodChek Systems 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

FOR FURTHER INFORMATION PLEASE CONTACT:
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