



Actero™ Listeria Enrichment Media* Product Information

INTENDED USE:

Actero™ Listeria Enrichment Media (“*Actero™ Listeria*”) is a selective medium specifically optimized for a 18-30 hours single-step recovery and enrichment of *Listeria* spp. from food and environmental samples.

PRINCIPLE OF OPERATION:

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:

Actero™ Listeria Enrichment Media EZ-Media Dry Bag – two port 5 L (Cat# FCM-266) and 20 L (Cat# FCM-267) contains sufficient quantity of dehydrated powder to prepare 5 or 20 L of *Actero™ Listeria Enrichment Media*.

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) pre-moistened with neutralizing Dey-Engley buffer (D/E) (FoodChek Cat # FCLS-005).

PROCEDURE FOR EZ-MEDIA DRY BAG:

Materials not provided: The required ancillary tubing and connectors can be purchased separately.

Instructions: Observe aseptic techniques from media preparation through to dispensing. Remove the *Actero™ EZ-Media Dry Bag* from its packaging, unfold and lay it flat on the bench with tubing and caps facing up. There are two tubes on the *Actero™ EZ-Media Dry Bag* with red caps. The “in” port does not have a connector and the “out” port has a male connector to ensure sterility while the media is being prepared and later dispensed.

Aseptically remove the sterile filter from its separate bag. The filter has 2 male ends and there is an arrow on the filter to indicate the “in” and

“out”. Connect the “in” end of the filter nozzle to the pump or dilutor tubing (used for adding the deionized water to the *Actero™ EZ-Media Dry Bag*). We recommend securing this connection with a tie wrap or zip tie (not provided).

Next, remove the sterile cap from the “in” tube (without connector) attached to the *Actero™ EZ-Media Dry Bag* and connect it to the other end of the filter nozzle to permit the flow of deionized water into your tubing. Loosen the vent valve on the filter and slowly begin to fill the filter capsule with the deionized water so that the liquid reaches the level of the vent. As soon as all excess air escapes the capsule and the deionized water reaches the level of the vent, tighten the vent valve to close it. Then, gradually increase the flow rate or pressure of the pump or dilutor to the desired value (maximum flow rate: 350mL/min/0.1bar; maximum operating pressure: 4.1 bar).

Turn off your pump or dilutor when the corresponding volume of water (5L or 20L) has been added to the *Actero™ EZ-Media Dry Bag*. Remove the *Actero™ EZ-Media Dry Bag* tube from the filter nozzle and replace the cap on the tube end. Gently massage the *Actero™ EZ-Media Dry Bag* until the media is completely dissolved. The prepared media in the *Actero™ EZ-Media Dry Bag* can be stored up to 7 days at room temperature on the bench.

The supplied filter is intended to be used only once or, if necessary, when rehydrating several bags within a short amount of time, as long as the manipulation is done aseptically. Otherwise, disconnect the filter from the pump or dilutor tubing and allocate the filter for biohazard waste disposal according to local, municipal, provincial, state and/or federal regulations.

When ready to use the prepared media, remove the cap from the “out” tube (with connector) on the *Actero™ EZ-Media Dry Bag*, aseptically connect it to your dispensing pump and dispense the appropriate volume into your sample bags. You can directly connect the dispensing pump tubing to the connector or use the female adapter available at FoodChek Systems Inc available under catalog number FCLM-028.

QUALITY CONTROL SPECIFICATIONS

1. The powder is homogeneous, free flowing, and beige.
2. Visually, the prepared medium is light to medium amber and with none to slight precipitate.

* All mentions of *Actero™ Listeria Enrichment Media* or *Actero™ Listeria* refer to the patented *Actero™ Elite* product for the detection of *Listeria* spp.



Function	Control strains	Incubation	Method of control	Criteria	Expected results
Productivity	<i>Listeria monocytogenes</i> 4b ATCC 13932 + <i>Escherichia coli</i> ATCC 25922 + <i>Enterococcus faecalis</i> ATCC 19433	18h / 37°C	Qualitative	> 100 colonies on Agar <i>Listeria</i> according to Ottaviani and Agosti	Blue green colonies with opaque halo
	<i>Listeria monocytogenes</i> 1/2a ATCC 35152 + <i>Escherichia coli</i> ATCC 25922 + <i>Enterococcus faecalis</i> ATCC 19433				
	<i>Listeria grayi</i> ATCC 19120 + <i>Escherichia coli</i> ATCC 25922 + <i>Enterococcus faecalis</i> ATCC 19433				
	<i>Listeria grayi</i> ATCC 25401 + <i>Escherichia coli</i> ATCC 25922 + <i>Enterococcus faecalis</i> ATCC 19433				
Selectivity	<i>Escherichia coli</i> ATCC 25922	24h / 37°C	Qualitative	Total inhibition (0) on TSA	-
	<i>Enterococcus faecalis</i> ATCC 19433		Qualitative	< 100 colonies on TSA	-

The performance test is in accordance with the current version of ISO 11133.

PROCEDURE

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.

Environmental Surface Sample Preparation

- Use a non-bactericidal sterile cellulose sampling sponge (8×4×0.3 cm) that has been pre-moistened with D/E buffer.
- 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.
- 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.
- When ready to test, pre-warm the prepared *Actero™ Listeria* Enrichment Media at 29, 32 or 35°C depending on the detection method to be used.
- Add 90 mL of the pre-warmed *Actero™ Listeria* Enrichment Media to each sponge sample in its sample bag.

- 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 **20-24 h at 35 ± 2°C**.

Note: 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	<ol style="list-style-type: none"> 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. Homogenize sponge with 90 mL pre-warmed (29°C or 32°C) Actero™ <i>Listeria</i> Enrichment Media. Incubate at 29 ± 0.5°C for 24 h or at 32 ± 0.5°C for 28 h. 	<ul style="list-style-type: none"> ✓ By FoodChek™ <i>Listeria</i> spp. Assay ✓ By Actero™ <i>Listeria</i> Culture Method
	<ol style="list-style-type: none"> 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. Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ <i>Listeria</i> Enrichment Media. Incubate at 35 ± 2°C for 20-24 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ <i>Listeria</i> Culture Method



Non Food Contact Surface ➤ Rubber ➤ Sealed concrete ➤ Ceramic	<ol style="list-style-type: none"> Swab a 100 cm² surface with a sponge and keep it in a sterile bag at 4°C until tested. Homogenize sponge with 90 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. Incubate at 32 ± 0.5°C for 28 h. 	<ul style="list-style-type: none"> ✓ By FoodChek™ Listeria spp. Assay ✓ By Actero™ Listeria Culture Method
Non Food Contact Surface ➤ Sealed concrete	<ol style="list-style-type: none"> Swab a 100 cm² surface with a sponge and keep it in a sterile bag at 4°C until tested. Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 20-24 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ 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	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. Incubate at 32 ± 0.5°C for 28 h. 	<ul style="list-style-type: none"> ✓ By FoodChek™ Listeria spp. Assay ✓ By Actero™ Listeria Culture Method
➤ Cold smoked salmon	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (29°C) Actero™ Listeria Enrichment Media. Incubate at 29 ± 0.5°C for 28 h. 	<ul style="list-style-type: none"> ✓ By FoodChek™ Listeria spp. Assay ✓ By Actero™ Eite Listeria Culture Method
	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 22-24 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Frozen cooked shrimp	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. Incubate at 32 ± 0.5°C for 28 h. 	<ul style="list-style-type: none"> ✓ By FoodChek™ Listeria spp. Assay ✓ By Actero™ Listeria Culture Method
	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 22-24 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Raw frozen salmon	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 100 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 18-22 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ 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)	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. Incubate at 32 ± 0.5°C for 28 h. 	<ul style="list-style-type: none"> ✓ By FoodChek™ Listeria spp. Assay ✓ By Actero™ Listeria Culture Method
➤ Mexican-style cheese	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 22-24 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Ice cream	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 18-22 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ 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	<ol style="list-style-type: none"> Homogenize (30 sec) 25 g sample with 100 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 0.5°C for 22-24 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
	<ol style="list-style-type: none"> Homogenize (30 sec) 25 g sample with 150 mL pre-warmed (32°C) Actero™ Listeria Enrichment Media. Incubate at 32 ± 0.5°C for 28 h. 	<ul style="list-style-type: none"> ✓ By Actero™ Listeria Culture Method
➤ Bagged fresh spinach	<ol style="list-style-type: none"> Homogenize (30 s) 25 g sample with 150 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 22-24 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Hot dog sausage	<ol style="list-style-type: none"> Homogenize (30 s) 125 g sample with 750 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 26-28 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Smoked turkey breast	<ol style="list-style-type: none"> Homogenize (30 s) 125 g sample with 500 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 24-28 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method
➤ Cured ham	<ol style="list-style-type: none"> Homogenize (30 s) 125 g sample with 500 mL pre-warmed (35°C) Actero™ Listeria Enrichment Media. Incubate at 35 ± 2°C for 26-28 h. 	<ul style="list-style-type: none"> ✓ By DuPont™ BAX® System Real-Time PCR Assays for Genus <i>Listeria</i> and <i>L. monocytogenes</i> ✓ By Actero™ Listeria Culture Method



Food Sample Preparation

Liquid Whole Eggs (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 **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.

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.

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** 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 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.

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 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),

DuPont™ 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 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 *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)

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 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**.

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.

DuPont™ 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)

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

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).

FoodChek™ Listeria spp. Assay – All samples

Refer to the Analysis section of the package insert for the FoodChek™ 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:

On receipt, store the EZ-Media Dry Bag at room-temperature 15-25°C. The expiry date is indicated on the package.

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/bmb15/bmb1.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 NUMBERS:

FCM-259: Actero™ Listeria Enrichment EZ-Media Dry Bag, 15 x 5 L
FCM-257: Actero™ Listeria Enrichment EZ-Media Dry Bag, 12 x 20 L

FOR FURTHER INFORMATION, PLEASE CONTACT:

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