



Actero™ Salmonella Enrichment Media Product Information

INTENDED USE:

Actero™ Salmonella Enrichment Media is a selective medium optimized for an improved enrichment of *Salmonella* spp. from food and environmental surface samples.

PRINCIPLE OF OPERATION:

The principle of Actero™ Salmonella Enrichment Media is based on the ability of *Salmonella* strains to optimized growth by the use of specific nutrients that are contained within the Actero™ media. This unique medium formulation confers an important growth advantage when other bacteria are present.

CONTENTS:

Actero™ MediaBox Salmonella Enrichment Media 5 L (Cat# FCM-047) and 10 L (Cat# FCM-048) contains respectively 5 L and 10 L of ready-to-use Actero™ Salmonella Enrichment Media.

ADDITIONAL MATERIALS REQUIRED:

1. Distilled/deionized, sterile water.
2. Sterile filter and non-filter Stomacher® bags.
3. Serological pipette, sterile.
4. Water bath 39-40 ± 0.5°C
5. Incubator: at 39 ± 0.5°C, 35 ± 2°C
6. Tips and Adjustable Volume Pipette (100 - 1000 µL).
7. 10 µL calibrated inoculating loop
8. Rappaport-Vassiliadis Broth (RV).
9. Tetrathionate broth.
10. Xylose Lysine Tergitol-4 Agar (XLT4)
11. BG Sulfa Agar (BGS)
12. Xylose Lysine Deoxycholate agar (XLD)
13. Hektoen Enteric agar (HE)
14. Stomacher® 3500/Stomacher® 400 (optional) available from multiple sources or equivalent.
15. Other regular laboratory equipment could also be 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 MEDIABOX:

Materials not provided:

Ancillary tubing and connectors required can be purchased separately, see list of accessory tubing and connectors itemized later in the instructions for use.

Instructions:

Observe aseptic techniques. Stand the MediaBox with the Cap and dispensing tube at the top.

Set-up your dispensing tubing by connecting it to a pump or dilutor you use to control dispensing of the broth.

Have your sterile connecting tubing with suitable connector ready to link to the MediaBox. Remove the sterile caps from both the dispensing tube attached to the MediaBox and the sterile connector stopper to your dispensing tubing. Connect the two pieces to permit flow of the broth into your dispensing tubing. Turn the MediaBox on its side with the dispensing cap and tubing toward the bottom of the MediaBox on the bench. Turn on your pump or dilutor and commence dispensing the broth into blender bags, bottles, tubes or other suitable vessels.

User Quality Control:

1. Examine initial dispensed broth from the MediaBox to confirm that the liquid is not cloudy, as this could indicate bacteria contamination in the MediaBox.
2. Inspect the MediaBox upon receipt for any signs of dampness on the outer box as this could indicate leakage of broth during transport.

Summary of Enrichment and Analysis of Samples Using Actero™ Salmonella 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 the sample with 90 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media. 3. Incubate at 39 ± 0.5°C for 18 h.	✓ By Actero™ Salmonella Method ✓ By FoodChex™ Salmonella Assay
	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 the sample with 90 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. 3. Incubate at 35 ± 2°C for 14 - 18 h.	✓ By DuPont™ BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Method
Non Food Contact Surface ➤ Rubber ➤ Sealed concrete ➤ Ceramic	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 the sample with 90 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media. 3. Incubate at 39 ± 0.5°C for 18 h.	✓ By Actero™ Salmonella Method ✓ FoodChex™ Salmonella Assay
Sample Type	Sample Preparation	Analysis of Enriched Samples
Food Sample		
Ground Chicken	1. Homogenize (30 s) 25 g sample with 50 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media. 2. Incubate at 39 ± 0.5°C for 20 h in an incubator.	✓ By Actero™ Salmonella Culture Method



Ground Beef	<ol style="list-style-type: none"> 1. Homogenize (30 s) 325 g sample with 650 mL pre-warmed (39°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 39.5 ± 0.5°C for 7 h in a water bath 3. Transfer 0.5 mL of enriched sample into 10 mL TBG and 0,1 mL into 10 mL RVS and incubate at 42 ± 0.5°C for 22-24 h in a water bath. 	✓ By Actero TM Salmonella Culture Method
Whole Liquid Egg	<ol style="list-style-type: none"> 1. Homogenize (30 s) 100 g sample with 300 mL pre-warmed (39°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 39 ± 0.5°C for 7 h in a water bath. 	✓ By Actero TM Salmonella Culture Method
	<ol style="list-style-type: none"> 1. Homogenize (30 s) 100 g ± 2 g sample with 700 mL pre-warmed (39°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 39 ± 0.5°C for 18 h in an incubator. 	<ul style="list-style-type: none"> ✓ By ActeroTM Salmonella Culture Method ✓ By FoodChekTM Salmonella Assay
Raw Frozen Scallop	<ol style="list-style-type: none"> 1. Homogenize (30 s) 25 g sample with 50 mL pre-warmed (39°C) ActeroTM Salmonella Enrichment Media. 2. For water bath: Incubate at 39 ± 0.5°C for 7 h. For incubator: Incubate at 39 ± 0.5°C for 18 h. 	✓ By Actero TM Salmonella Culture Method
Sprout	<ol style="list-style-type: none"> 1. Homogenize (60 s) 25 g sample with 150 mL pre-warmed (39°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 39 ± 0.5°C for 7 h in a water bath. 3. Transfer 1,0 mL of enriched sample into 10 mL TBG and 0,1 mL into 10 mL RVS and incubate respectively at 43 ± 0.2°C C and at 42 ± 0.2°C for 18 h in water bath. 	✓ By Actero TM Salmonella Culture Method
Milk Chocolate	<ol style="list-style-type: none"> 1. Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 22-26 h. 	<ul style="list-style-type: none"> ✓ By DuPontTM BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By ActeroTM Salmonella Culture Method
	<ol style="list-style-type: none"> 1. Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 39 ± 0.5°C for 18 h. 	✓ By Actero TM Salmonella Culture Method
Chocolate Liqueur	<ol style="list-style-type: none"> 1. Homogenize (60 s) 25 g sample with 225 mL pre-warmed (35°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 26-30 h. 	<ul style="list-style-type: none"> ✓ By DuPontTM BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By ActeroTM Salmonella Culture Method
Dry Pet Food	<ol style="list-style-type: none"> 1. Homogenize (60 s) 25 g sample with 225 mL pre-warmed (35°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 18-22 h. 	<ul style="list-style-type: none"> ✓ By DuPontTM BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By ActeroTM Salmonella Culture Method
	<ol style="list-style-type: none"> 1. Homogenize (60 s) 375 g sample with 2625 mL pre-warmed (35°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 18-22 h. 	<ul style="list-style-type: none"> ✓ By DuPontTM BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By ActeroTM Salmonella Culture Method
Shell Egg	<ol style="list-style-type: none"> 1. Homogenize by hands 20 egg sample with 1000 mL pre-warmed (35°C) ActeroTM Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 16-20 h. 	<ul style="list-style-type: none"> ✓ By DuPontTM BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By ActeroTM Salmonella Culture Method

Environmental Surface Sample Preparation

ActeroTM Salmonella Culture Method or FoodChekTM Salmonella Assay

1. Add to the non-bactericidal, non-bacteriostatic 8×4×0.3 cm sterile cellulose sampling sponge pre-moistened with D/E.
2. Wipe the surface to be tested with one side of the sponge (with excess liquid gently squeezed out) 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 cover 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 ActeroTM Salmonella Enrichment Media at $35 \pm 2^{\circ}\text{C}$ or $39 \pm 0.5^{\circ}\text{C}$.
5. Add $90 \pm 5 \text{ mL}$ of the pre-warmed ActeroTM Salmonella Enrichment Media to each sponge sample in its sample bag.
6. Homogenize the sample for **30 seconds** in a Stomacher[®] 400 or equivalent. Hand mixing, is an acceptable alternative for stomaching. To hand mix, massage each sponge that is in the sealed Stomacher[®] bag for approximately 1 minute.

Enrichment of Environmental Surface Samples Using ActeroTM Salmonella Enrichment Media

Stainless Steel, Plastic

ActeroTM Salmonella Culture Method or FoodChekTM Salmonella Assay

For the enrichment phase, close the bag and incubate the sample in an incubator for $18 \pm 0.5 \text{ h}$ at $39 \pm 0.5^{\circ}\text{C}$. Adherence to temperature is important for accurate results.

DuPontTM BAX[®] System Real-Time PCR Assays for *Salmonella* or ActeroTM Salmonella Culture Method

For the enrichment phase, close the bag loosely and incubate the sample in an incubator for $16 \pm 2 \text{ h}$ at $35 \pm 2^{\circ}\text{C}$.

Rubber, Ceramic Tile and Sealed Concrete

ActeroTM Salmonella Culture Method or FoodChekTM Salmonella Assay

For the enrichment phase, close the bag and incubate the sample in an incubator for $18 \pm 0.5 \text{ h}$ at $39 \pm 0.5^{\circ}\text{C}$. Adherence to temperature is important for accurate results.

At the end of the enrichment period, mix sample thoroughly and transfer $10.0 \pm 0.1 \text{ mL}$ of the enriched sample to a tube. Cap the tube.

Preparation and Enrichment of Food Samples Using ActeroTM Salmonella Enrichment Media

Raw Ground Chicken (25 g)

ActeroTM Salmonella Culture Method

1. Add **50 mL** of pre-warmed (39°C) and supplemented ActeroTM Salmonella broth to **25 g** of sample in a filter-equipped Stomacher[®] bag.
2. Homogenize the sample for **30 seconds** in a Stomacher[®] 400 circulator or equivalent or mix vigorously in the Stomacher[®] bag for 1 minute if there is no Stomacher[®] machine available.
3. Close bag loosely and incubate the sample upright for **20 h at 39°C** in an **incubator** for enrichment.
4. After 20 hours, remove the sample from the incubator, re-suspend the contents by shaking the bag and transfer $10.0 \pm 0.1 \text{ mL}$ to a tube. Cap the tube.

Raw Ground Beef (325 g)

ActeroTM Salmonella Culture Method

1. Add **650 mL** of pre-warmed and supplemented ActeroTM Salmonella broth to **325 g** of sample in a filter-equipped Stomacher[®] bag.



2. Homogenize the sample for **30 seconds** in a Stomacher® 3500 or equivalent. Alternatively, mix vigorously in bag for 1 minute if there is no Stomacher® machine available.
3. Close bag loosely and incubate the samples for **7 h at 39.5°C** in a water bath for enrichment. If there are a large number of samples to be analyzed, verify that the temperature of the water between the sample bags reaches 39.5°C before starting to record the required incubation time. It is important to precisely control the enrichment period to obtain valuable accurate results.
4. After 7 hours, remove the sample from the water bath, mix the contents by shaking the bag and transfer **10.0 ± 0.1 mL** to a tube. Cap the tube.
5. Transfer **0.5 ml** of enriched sample into **10 mL Tetrathionate Broth** and **0.1 ml into 10 mL modified Rappaport-Vassiliadis broth** and incubate tubes at **42 ± 0.5°C for 22-24 h**.

Note: Test limitation: test standardization is for meat with a maximum aerobic microbial flora of 4×10^5 cfu/g

Whole Liquid Egg (100 g)

Actero™ Salmonella Culture Method: 7 h Enrichment in Water Bath

1. Add **300 ml** of pre-warmed (**39°C**) and supplemented Actero™ Salmonella broth to **100 g** of sample in a filter-equipped Stomacher® bag. Adjust pH, if necessary, to 7.0 ± 0.4 .
2. Homogenize the sample for **30 seconds at 150 rpm** in a Stomacher® 3500 or equivalent. Alternatively, mix in bag until homogeneous if there is no Stomacher® machine available.
3. Close bag loosely and incubate the sample upright for **7 h at 39°C** in a **water bath** for enrichment. If there are a large number of samples to be analyzed, verify that the temperature of the water between the sample bags reaches 39°C before starting to record the required incubation time. It is important to precisely control the enrichment period to obtain valuable accurate results.
4. After 7 hours, remove the sample from the water bath, mix the contents by shaking the bag and transfer **10.0 ± 0.1 mL** to a tube. Cap the tube.

Actero™ Salmonella Culture Method or FoodChex™ Salmonella assay: 18 h Enrichment in Incubator

1. Add **700 ml** of pre-warmed (**39°C**) and supplemented Actero™ Salmonella broth to **100 g** of sample in a non-filtered Stomacher® bag. Adjust pH, if necessary, to 7.0 ± 0.4 .
2. Homogenize the sample for **30 seconds** in a Stomacher® 3500 or equivalent. Alternatively, mix vigorously in bag for 1 minute if there is no Stomacher® machine available.
3. Close bag loosely and incubate the sample upright for **18 h at 39°C** in an **incubator** for enrichment.
4. After 18 hours, remove the sample from the incubator, mix the contents by shaking the bag and transfer **10.0 ± 0.1 mL** to a tube. Cap the tube.

Raw Frozen Scallop (25 g)

Actero™ Salmonella Culture Method

1. Add **50 ml** of pre-warmed (**39°C**) and supplemented Actero™ Salmonella broth to **25 g** of sample in a filter-equipped Stomacher® bag.
2. Homogenize sample for **30 seconds** in a Stomacher® 400 circulator or equivalent. Alternatively, mix vigorously in bag for 1 minute if there is no Stomacher® machine available.

For a 7 h Enrichment in Water Bath

3. Close bag loosely and incubate sample upright for **7 h at 39°C** in a **water bath** for enrichment. If there are a large number of samples to be analyzed, verify that the temperature between the sample bags reaches 39°C



before starting to record the incubation time. It is important to precisely control the enrichment period to obtain valuable and accurate results.

4. After 7 hours, remove the samples from the water bath, mix the contents by shaking the bag and transfer **10.0 ± 0.1 mL** to a tube. Cap the tube.

For a 18 h Enrichment in Incubator

5. Close bag loosely and incubate sample upright for **18 h at 39°C** in an **incubator** for enrichment.
6. After 18 hours, remove the samples from the incubator, mix the contents by shaking the bag and transfer **10.0 ± 0.1 mL** to a tube. Cap the tube

Sprout (25 g)

Actero[™] Salmonella Culture Method

1. Add **150 ml** of pre-warmed (**39°C**) Actero[™] Salmonella broth to **25 g** of sample in a filter-equipped Stomacher[®] bag.
2. Homogenize sample for **60 seconds** in a Stomacher[®] 400 circulator or equivalent. Alternatively, mix vigorously in bag for 1 minute if there is no Stomacher[®] machine available.
3. Close bag loosely and incubate sample upright for **7 h at 39°C** in a **water bath** for enrichment. If a large number of samples are to be analyzed, verify that the temperature between the sample bags reaches 39°C before starting to record the incubation time. It is important to precisely control the enrichment period to obtain valuable and accurate results.
4. After 7 hours, remove the sample from the water bath, mix the contents by shaking the bag and transfer **10.0 ± 0.1 mL** to a tube. Cap the tube
5. Transfer **1.0 ml** of enriched sample into **10 mL Tetrathionate Broth** and **0.1 ml** into **10 mL Rappaport-Vassiliadis broth (RV)** and incubate respectively at **43 ± 0.2°C** and **42 ± 0.2°C** for **18 h** (because sprouts are considered to have high microbial load).

Dry Pet Food

DuPont[™] BAX[®] System Real-Time PCR Assay for *Salmonella* or Actero[™] Salmonella Culture Method

1. Add **225 ± 5 mL** of pre-warmed (**35 ± 2°C**) Actero[™] Salmonella broth to **25 g** of sample in a filter-equipped Stomacher[®] bag.
2. Add **2625 ± 25 mL** of pre-warmed Actero[™] Salmonella broth to **375 g** of sample in a filter-equipped Stomacher[®] bag.
3. Homogenize each sample for **60 seconds** in a Stomacher[®] 400 circulator or Stomacher[®] 3500 or equivalent.
4. Close the bag loosely, and incubate the sample for **18-22 h** at **35 ± 2°C** using an incubator.

Milk Chocolate

1. Pre-warm the Actero[™] Salmonella medium at before use.
2. Add **175 ± 5 mL** of pre-warmed (**35 ± 2°C**) Actero[™] Salmonella broth to **25 g** of sample in a filter-equipped Stomacher[®] bag.
3. Homogenize each sample for **60 seconds** in a Stomacher[®] 400 circulator or equivalent.

DuPont[™] BAX[®] System Real-Time PCR Assay for *Salmonella*

4. Close the bag loosely, and incubate the sample for **22-26 h** at **35 ± 2°C** using an incubator.

Actero[™] Salmonella Culture Method

5. Close the bag loosely, and incubate the sample for **18-22 h** at **39 ± 0.5°C** or **22-26 h** at **35 ± 2°C** using an incubator.



Chocolate Liquor

DuPontTM BAX[®] System Real-Time PCR Assay for *Salmonella* or ActeroTM Salmonella Culture Method

1. Add **225 ± 5 mL** of pre-warmed (**35 ± 2°C**) ActeroTM Salmonella broth to **25 g** of sample in a filter-equipped Stomacher[®] bag.
2. Homogenize each sample for **2 minutes** at **175 rpm** in a Stomacher[®] 400 circulator or equivalent.
3. Close the bag loosely, and incubate the sample for **26-30 h** at **35 ± 2°C** using an incubator.

Shell Egg

DuPontTM BAX[®] System Real-Time PCR Assay for *Salmonella* or ActeroTM Salmonella Culture Method

1. Add **1000 ± 50 mL** of pre-warmed (**35 ± 2°C**) ActeroTM Salmonella broth to each filter bag containing the 20-egg test portion.
2. Homogenize each sample by hand mixing.
3. Close the bag loosely, and incubate the sample for **16-20 h** at **35 ± 2°C** using an incubator

Analysis of Enriched Samples

ActeroTM Salmonella Culture Method

Raw ground chicken, raw ground beef whole liquid eggs:

- ✓ Streak the samples onto selective agar plates (XLT4 and BGS) using a calibrated loop of 10 µL and, if necessary, follow the confirmation procedure as recommended in the USDA FSIS Microbiology Laboratory Guidebook Chapter 4.08.

Raw frozen scallop, sprouts, dry pet food, milk chocolate, chocolate liquor, environmental surface samples:

- ✓ Streak the samples onto selective agar plates (XLD and HE) using a calibrated loop of 10 µL and, if necessary, follow the confirmation procedure as recommended in the US FDA Bacteriological Analytical Manual Chapter 5.

Interpretation and Test Result Report

- ✓ All samples presenting typical colony (ies) after 24 or 48 h in the selective agar should be consider as presumptive positive(s). The presumptive results confirmed according the US FDA Bacteriological Analytical Manual Chapter 5 and USDA FSIS Microbiology Laboratory Guidebook Chapters 4.08.
- ✓ All samples which do not present typical colonies after 48h of incubation can be consider as negative samples.

DuPontTM BAX[®] System Real-Time PCR Assay for *Salmonella*

Enriched Sample Preparation Prior to Testing

1. Label and arrange a 2 mL microcentrifuge tubes in a rack.
2. Add **2 mL** of PBS to each microcentrifuge tube and place it with an open cap in a microcentrifuge tube rack.
 - a. For dry pet food, milk chocolate and chocolate liquor transfer **80 µL** enriched sample from the bag into each microcentrifuge tube.
 - b. For cocoa powder, shell egg, stainless steel and plastic environmental sponge samples, transfer **40 µL** enriched sample from the bag into each microcentrifuge tube.



3. Refer to the Test Protocol section of the package insert for the DuPont[™] BAX[®] System Real-Time PCR Assay for *Salmonella* for the following steps.

FoodChek[™] Salmonella Assay

Refer to the Analysis section of the package insert for the FoodChek[™] Salmonella assay.

PRODUCT STORAGE AND SHELF LIFE:

On receipt, store the MediaBox at 2-8°C. The expiry date is indicated on the package.

DISPOSAL:

Dispose all materials used and the enrichment media by autoclaving or according to an approved practice. Ensure that all biohazardous waste is disposed of according to local, municipal, provincial, state and/or federal regulations.

PRECAUTIONS:

Salmonella are categorized as Biosafety Level 2 pathogens. Biosafety level 2 procedures should be exercised (BMBL, <http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf>). The use of microbiological media such as the Actero[™] Salmonella Enrichment Media requires trained laboratory personnel familiar with good microbiological laboratory practices. Wear a laboratory coat, disposable gloves and eye protection while handling specimens and performing the assay is strongly recommended. Material Safety Data Sheet (MSDS) must be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis. The personnel who will handle the material should read the MSDS prior to start-up.

All enrichment broths may contain various pathogens whether they contain *Salmonella* spp. or not. Furthermore, some pathogen bacteria have a very low infective dose (Ex. *E. coli* O157:H7 is estimated to be 50 organisms). Thus, extreme care should be taken in handling test samples and enrichment broths.

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-047: Actero[™] MediaBox Salmonella Enrichment Media (5 L)

FCM-048: Actero[™] MediaBox Salmonella Enrichment Media (10 L)



FOR FURTHER INFORMATION PLEASE CONTACT:

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