



Actero™ MediaBox™ 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) contain 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. Rapid™ Salmonella
15. CHROMagar™
16. Nonfat Dry Milk (NFD)
17. Malachite green
18. Stomacher® 3500/Stomacher® 400 (optional) available from multiple sources or equivalent.
19. Other regular laboratory equipment could also be required.

Environmental Samples

Non-bactericidal sterile cellulose sampling sponges (8×4×0.3 cm) pre-moistened with neutralizing Dey-Engley buffer (D/E). (Salus Scientific Inc. 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 a 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.
3. Verify that the pH of the MediaBox™ is 8.2 ± 0.2.

Environmental Surface Sample Preparation

Actero™ Salmonella Culture Method

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 Actero™ Salmonella Enrichment Media at 35 ± 2°C or 39 ± 0.5°C.
5. Add 90 ± 5 mL of the pre-warmed Actero™ 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 Actero™ Salmonella Enrichment Media

Stainless Steel, Plastic

Actero™ Salmonella Culture Method

For the enrichment phase, close the bag and incubate the sample in an incubator for 18 ± 0.5 h at 39 ± 0.5°C. Adherence to temperature is important for accurate results.

BAX® System Real-Time PCR Assays for *Salmonella* or Actero™ Salmonella Culture Method

For the enrichment phase, close the bag loosely and incubate the sample in an incubator for 16 ± 2 h at 35 ± 2°C



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 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 the sample with 90 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media . 3. Incubate at 35 ± 2°C for 14 - 18 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture 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 Culture Method
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 .	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) 25 g sample with 225 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media supplemented with 50 mg/L malachite green. 2. Incubate at 35 ± 2.0°C for 14-18 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Chicken Carcass Rinse	1. Homogenize (30 s) 30 mL sample with 30 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media supplemented with 20 mg/L malachite green. 2. Incubate at 35 ± 2.0°C for 16-20 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Ground Beef	1. Homogenize (30 s) 325 g sample with 650 mL pre-warmed (39°C) Actero™ 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™ Salmonella Culture Method
	1. Homogenize (60 s) 25 g sample with 75 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media supplemented with 50 mg/L malachite green. 2. Incubate at 35 ± 2.0°C for 16-20 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
	1. Homogenize (60 s) 375 g sample with 1125 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media supplemented with 25 mg/L malachite green. 2. Adjust pH to 7.0 ± 0.2 if necessary. 3. Incubate at 39 - 42 ± 0.5°C for 20-24 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Whole Liquid Egg	1. Homogenize (30 s) 100 g sample with 300 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media . 2. Incubate at 39 ± 0.5°C for 7 h in a water bath.	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) 100 g ± 2 g sample with 700 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media . 2. Incubate at 39 ± 0.5°C for 18 h .	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) 100 g ± 2 g sample with 300 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media . 2. Adjust pH to 7.0 ± 0.2 if necessary. 3. Incubate at 35 ± 2.0°C for 18-22 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Dried Whole Egg	1. Homogenize (30 s) 100 g sample with 300 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media supplemented with 5% NFD. M . 2. Incubate at 35 ± 2.0°C for 14-18 h .	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) 100 g sample with 600 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media supplemented with 5% NFD. M . 2. Incubate at 35 ± 2.0°C for 14-18 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Shell Egg	1. Homogenize by hand a sample of 20 eggs with 1000 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media . 2. Incubate at 35 ± 2°C for 16-20 h .	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Raw Frozen Scallop	1. Homogenize (30 s) 25 g sample with 50 mL pre-warmed (39°C) Actero™ 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™ Salmonella Culture Method



Sample Type	Sample Preparation	Analysis of Enriched Samples
Sprout	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 150 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media. Incubate at 39 ± 0.5°C for 7 h in a water bath. 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 a water bath. 	<ul style="list-style-type: none"> ✓ By Actero™ Salmonella Culture Method
Milk Chocolate	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 22-26 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 39 ± 0.5°C for 18 h. 	<ul style="list-style-type: none"> ✓ By Actero™ Salmonella Culture Method
Chocolate Liquor	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 225 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 26-30 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Cocoa Powder	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media supplemented with 5% NFDN. Incubate at 35 ± 2°C for 16-20 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Dry Pet Food	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 225 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 18-22 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
	<ol style="list-style-type: none"> Homogenize (60 s) 375 g sample with 2625 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 18-22 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Raw Almond	<ol style="list-style-type: none"> Homogenize (60 s) 375 g sample with 750 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 16-20 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Peanut Butter	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 16-20 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Dried Parsley	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 225 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media then manually to remove parsley from the bag walls. Incubate at 35 ± 2°C for 20-24 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Dried Raisin	<ol style="list-style-type: none"> Homogenize (60 s) 25 g sample with 75 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 16-20 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Whole Black Pepper	<ol style="list-style-type: none"> Homogenize 25 ± 1 g sample manually with 75 ± 5 mL of pre-warmed Actero™ Salmonella Enrichment Media. Incubate at 35 ± 2°C for 16 -20 h. 	<ul style="list-style-type: none"> ✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method

Rubber, Ceramic Tile and Sealed Concrete

Actero™ Salmonella Culture Method

For the enrichment phase, close the bag and incubate the sample in an incubator for 18 ± 0.5 h at 39 ± 0.5°C. Adherence to temperature is important for accurate results.

At the end of the enrichment period, mix the sample thoroughly and transfer 10.0 ± 0.1 mL of the enriched sample to a tube. Cap the tube.

Preparation and Enrichment of Food Samples Using Actero™ Salmonella Enrichment Media

Raw Ground Chicken (25 g)

Actero™ Salmonella Culture Method

- Add 50 mL of pre-warmed (39°C) and supplemented Actero™ Salmonella broth to 25 g of sample in a filter-equipped Stomacher® bag.
- 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.
- Close the bag loosely and incubate the sample upright for 20 h at 39°C in an incubator for enrichment.
- After 20 hours, remove the sample from the incubator, re-suspend the contents by shaking the bag and transfer 10.0 ± 0.1 mL to a tube. Cap the tube.

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

- Pre-warm the Actero™ Salmonella broth supplemented with 50 mg/L malachite green at 35 ± 2°C before use.
- Add 225 ± 5 mL of pre-warmed Actero™ Salmonella broth to each filter bag containing the 25 ± 1 g test portion.
- Mix each sample by hand to homogenize it.
- Close the bag loosely, and incubate the sample for 14 - 18 h at 35 ± 2°C using an incubator.

Chicken Carcass Rinse

BAX® System Real-Time PCR Assays for *Salmonella* or Actero™ Salmonella Culture Method

- Pre-warm the Actero™ Salmonella broth supplemented with 20 mg/L malachite green at 35 ± 2°C before use.
- Add 30 ± 1 mL of pre-warmed Actero™ Salmonella broth to each filter bag containing the 30 ± 1 mL test portion.
- Mix each sample by hand to homogenize it.
- Close the bag loosely, and incubate the sample for 16 – 20 h at 35 ± 2°C using an incubator.

Raw Ground Beef (325 g)

Actero™ Salmonella Culture Method

- Add 650 mL of pre-warmed and supplemented Actero™ 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 the bag for 1 minute if there is no Stomacher® machine available.
3. Close the 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

Raw Ground Beef (25 g)

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

1. Pre-warm the Actero™ Salmonella broth supplemented with 50 mg/L malachite green at **39-42 ± 0.5°C** before use.
2. Add **75 ± 5 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **25 ± 1 g** test portion.
3. Homogenize each sample for 60 s using a stomacher.
4. Close the bag loosely, and incubate the **25 g sample for 16 - 20 h at 35 ± 2°C** using an incubator.

Raw Ground Beef (375 g)

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

1. Pre-warm the Actero™ Salmonella broth supplemented with 25 mg/L malachite green at **39 ± 0.5°C** before use.
2. Add **1125 ± 25 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **375 ± 10 g** test portion.
3. Homogenize each sample for 60 s using a stomacher.
4. Adjust pH to 7.0 ± 0.2 .
5. Close the bag loosely, and incubate the **375 g for 20 – 24 h at 39 ± 0.5°C** using an incubator.

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 the bag until homogeneous if there is no Stomacher® machine available.
3. Close the 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: 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 the bag for 1 minute if there is no Stomacher® machine available.
3. Close the 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.

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

1. Add **300 ml** of pre-warmed (**35°C**) and supplemented Actero™ Salmonella broth to **100 g** of sample in a non-filtered Stomacher® bag.
2. Mix vigorously by hand in the bag to homogenize it.
3. Adjust pH to 7.0 ± 0.2 .
4. Close the bag loosely and incubate the sample upright for 18-22 h at **35°C** in an incubator for enrichment.

Shell Egg

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

1. Add **1000 ± 50 mL** of pre-warmed (**35 ± 2°C**) Actero™ 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

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 the sample for **30 seconds** in a Stomacher® 400 circulator or equivalent. Alternatively, mix vigorously in the bag for 1 minute if there is no Stomacher® machine available.

For a 7 h Enrichment in Water Bath

1. Close the 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 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.
2. 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 an 18 h Enrichment in an Incubator

1. Close the bag loosely and incubate the sample upright for **18 h at 39°C** in an **incubator** for enrichment.
2. 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 the sample for **60 seconds** in a Stomacher® 400 circulator or equivalent. Alternatively, mix vigorously in the bag for 1 minute if there is no Stomacher® machine available.
3. Close the bag loosely and incubate the 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.

- 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
- 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

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

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

Milk Chocolate

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

BAX® System Real-Time PCR Assay for Salmonella

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

Actero™ Salmonella Culture Method

- 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

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

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

Raw Almond

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

- Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
- Add **750 ± 25 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **375 ± 10 g** sample.
- Homogenize each sample for **60 s** using a stomacher.
- Close the bag loosely, and incubate the sample for **16 - 20 h** at **35 ± 2°C** using an incubator.

Peanut Butter

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

- Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
- Add **175 ± 5 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **25 ± 1 g** test portion.
- Homogenize each sample for **60 s** using a stomacher.
- Close the bag loosely, and incubate the sample for **16 - 20 h** at **35 ± 2°C** using an incubator.

Dried Parsley

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

- Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
- Add **225 ± 5 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **25 ± 1 g** test portion.
- Homogenize each sample for **60 s** using a stomacher and then mix manually to remove the dried parsley from the sides of the bags.
- Close the bag loosely, and incubate the sample for **20-24 h** at **35 ± 2°C** using an incubator.

Dried Raisin

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

- Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
- Add **75 ± 5 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **25 ± 1 g** test portion.
- Homogenize each sample for **60 s** using a stomacher.
- Close the bag loosely, and incubate the sample for **16 -20 h** at **35 ± 2°C** using an incubator.

Whole Black Pepper

BAX® System Real-Time PCR Assay for Salmonella or Actero™ Salmonella Culture Method

- Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
- Add **75 ± 5 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **25 ± 1 g** test portion.
- Homogenize each sample manually.
- Close the bag loosely, and incubate the sample for **16 -20 h** at **35 ± 2°C** using an incubator.

Analysis of Enriched Samples

Actero™ Salmonella Culture Method

Raw ground chicken (39°C), raw ground beef (325 g), dried whole eggs, 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 current USDA FSIS Microbiology Laboratory Guidebook chapter for *Salmonella*.

Raw ground chicken (35°C), chicken carcass rinse

- ✓ Streak the enriched sample directly onto Rapid[®]Salmonella and BGS agar plates using a calibrated loop of 10 µL and, if necessary, follow the confirmation procedure as recommended in the current USDA FSIS Microbiology Laboratory Guidebook chapter for *Salmonella*.

Raw ground beef (25 g)

- ✓ Streak the enriched sample directly onto CHROMAgar™ Salmonella and BGS agar plates using a calibrated loop of 10 µL and, if necessary, follow the confirmation procedure as recommended in the current USDA FSIS Microbiology Laboratory Guidebook chapter for *Salmonella*.

Raw ground beef (375 g)

- ✓ Streak the enriched sample directly onto Rapid[®]Salmonella and XLT4 agar plates using a calibrated loop of 10 µL and, if necessary, follow the confirmation procedure as recommended in the current USDA FSIS Microbiology Laboratory Guidebook chapter for *Salmonella*.

Raw frozen scallops, sprouts, dry pet food, milk chocolate, chocolate liquor, raw almond, peanut butter, dried raisin, dried parsley, whole black pepper, whole liquid egg, 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 considered as presumptive positive(s). The presumptive results should be confirmed according to the US FDA Bacteriological Analytical Manual Chapter 5 and the current USDA FSIS Microbiology Laboratory Guidebook chapter for *Salmonella*.
- ✓ All samples which do not present typical colonies after 48h of incubation can be considered negative.

BAX® System Real-Time PCR Assay for *Salmonella*

Enriched Sample Preparation Prior to Testing

1. Label and arrange a 2 mL microcentrifuge tube 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 raw ground beef, raw ground chicken, chicken carcass rinse, dried whole egg, whole liquid egg, dry pet food, milk chocolate, chocolate liquor, raw almond, peanut butter, dried raisin, dried parsley and whole black pepper, 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 BAX® System Real-Time PCR Assay for *Salmonella* for the following steps.

PRODUCT STORAGE AND SHELF LIFE:

On receipt, store the MediaBox at 15-25°C. The expiry date is indicated on the package.

DISPOSAL:

Dispose of 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 (<https://www.cdc.gov/labs/BMBL.html>). 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:

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-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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