

## Actero™ Salmonella/STEC Enrichment Media Product Information

### INTENDED USE:

Actero™ Salmonella/STEC Enrichment Media is a selective medium optimized for an improved enrichment of *Salmonella* spp. from food and environmental surface samples. Furthermore, the medium allows the enrichment of the Shiga toxin-producing *E. coli* (STEC) along or co-enriched with *Salmonella* in ground beef.

### PRINCIPLE OF OPERATION:

The principle of Actero™ Salmonella/STEC Enrichment Media is based on the ability of *Salmonella* strains to optimize 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.

### KIT CONTENTS:

The kit contains sufficient material to prepare 35 liters of liquid medium.

- ✓ Dehydrated Actero™ Salmonella/STEC Enrichment Media, bottle of 500 g.
- ✓ Actero™ Salmonella/STEC Supplement # 1, 2 bottles of 35 mL.
- ✓ Actero™ Salmonella/STEC Supplement # 2, 1 bottle of 17 mL.

### 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<sup>®</sup>Salmonella
15. CHROMagar™
16. Nonfat Dry Milk (NFDM)
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:

#### Actero™ Salmonella/STEC Enrichment Media Preparation

##### With the use of AUTOCLAVE

1. Always shake the 500 g dry powder medium container before each use.
2. Measure **14.2 g** of dry medium powder on the weight scale.

3. Suspend and mix this 14.2 g of the medium into a clean one-liter bottle of distilled water.
4. Sterilize it by autoclaving at 121°C for 15 min.
5. Cool to room temperature and store at room temperature or refrigerate until use.
6. Prior to use, the medium must be pre-warmed followed by the **addition of 2 mL of supplement number 1 and 500 µL of supplement number 2 per liter of the medium.**
7. The pH should be at 8.2 ± 0.2 prior to use.

##### Without the use of AUTOCLAVE

1. Always shake the 500 g dry powder medium container before each use.
2. Measure **14.2 g** of dry medium powder on the weight scale.
3. Suspend and mix this 14.2 g in one liter of pre-warmed sterile distilled water.
4. The medium equilibrated now requires the addition of **2 mL of supplement number 1 and 0.5 mL of supplement number 2 per liter of the medium.**
5. The medium prepared should be used **immediately.**
6. The pH should be at 8.2 ± 0.2 prior to use.

#### Actero™ Salmonella/STEC Enrichment with 5% Nonfat Dry Milk (NFDM) Media Preparation

1. Always shake the 500 g dry powder medium container before use.
2. Measure **14.2 g** of dry medium powder on the weight scale.
3. Suspend and mix this 14.2 g of the medium into a clean one-liter bottle of distilled water.
4. Add **50 g** of NFDM to the medium mixture.
5. Sterilize this bottle by autoclaving at 121°C for **6 min.**
6. Remove the broth from the autoclave as soon as possible and cool to room temperature using ice water bath. Store refrigerated until use.
7. Prior to use, the medium must be pre-warmed followed by the **addition of 2 mL of supplement number 1 and 500 µL of supplement number 2 per liter of the medium.**
8. The pH should be at 8.2 ± 0.2 prior to use.

#### 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 cm<sup>2</sup>.
3. Place each surface sampled sponge in a sterile sample bag, and keep it at 4 ± 2 °C until it is ready for testing. Analyze sample units as soon as possible after their reception in the laboratory.
4. When ready to test, pre-warm the prepared Actero™ 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.

## Summary of Enrichment and Analysis of Samples Using Actero™ Salmonella/STEC Enrichment Media

Sample Type	Sample Preparation	Analysis of Enriched Samples
<b>Environmental Sample</b>		
Food Contact Surface ➤ Stainless steel ➤ Plastic	1. Swab a 100 cm <sup>2</sup> 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 <b>90 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 3. Incubate at <b>39 ± 0.5°C for 18 h.</b>	✓ By Actero™ Salmonella Culture Method
	1. Swab a 100 cm <sup>2</sup> 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 <b>90 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media.</b> 3. Incubate at <b>35 ± 2.0°C for 14 - 18 h.</b>	✓ 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 <sup>2</sup> 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 <b>90 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 3. Incubate at <b>39 ± 0.5°C for 18 h.</b>	✓ By Actero™ Salmonella Culture Method
<b>Food Sample</b>		
Ground Chicken	1. Homogenize (30 s) <b>25 g</b> sample with <b>50 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 2. Incubate at <b>39 ± 0.5°C for 20 h.</b>	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) <b>25 g</b> sample with <b>225 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media</b> supplemented with 50 mg/L malachite green. 2. Incubate at <b>35 ± 2.0°C for 14-18 h.</b>	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Chicken Carcass Rinse	1. Homogenize (30 s) <b>30 mL</b> sample with <b>30 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media</b> supplemented with 20 mg/L malachite green. 2. Incubate at <b>35 ± 2.0°C for 16-20 h.</b>	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Ground Beef	1. Homogenize (30 s) <b>325 g</b> sample with <b>650 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 2. Incubate at <b>39.5 ± 0.5°C for 7 h</b> in a water bath 3. Transfer <b>0.5 mL</b> of enriched sample into <b>10 mL TBG</b> and <b>0,1 mL</b> into <b>10 mL RVS</b> and incubate at <b>42 ± 0.5°C for 22-24 h</b> in a water bath.	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (60 s) <b>25 g</b> sample with <b>75 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media</b> supplemented with 50 mg/L malachite green. 2. Incubate at <b>39 - 42 ± 0.5°C for 16-20 h.</b>	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
	1. Homogenize (60 s) <b>375 g</b> sample with <b>1125 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media</b> supplemented with 25 mg/L malachite green. 2. Adjust pH to 7.0 ± 0.2 if necessary. 3. Incubate at <b>39 ± 0.5°C for 20-24 h.</b>	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Whole Liquid Egg	1. Homogenize (30 s) <b>100 g</b> sample with <b>300 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 2. Incubate at <b>39 ± 0.5°C for 7 h</b> in a water bath.	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) <b>100 g ± 2 g</b> sample with <b>700 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 2. Incubate at <b>39 ± 0.5°C for 18 h.</b>	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) <b>100 g ± 2 g</b> sample with <b>300 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media.</b> 2. Adjust pH to 7.0 ± 0.2 if necessary. 3. Incubate at <b>35 ± 2.0°C for 18-22 h.</b>	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Dried Whole Egg	1. Homogenize (30 s) <b>100 g</b> sample with <b>300 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media</b> supplemented with 5% NFDM. 2. Incubate at <b>35 ± 2.0°C for 14-18 h.</b>	✓ By Actero™ Salmonella Culture Method
	1. Homogenize (30 s) <b>100 g</b> sample with <b>600 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media</b> supplemented with 5% NFDM. 2. Incubate at <b>35 ± 2.0°C for 14-18 h.</b>	✓ 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 <b>20 eggs</b> with <b>1000 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media.</b> 2. Incubate at <b>35 ± 2°C for 16-20 h.</b>	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Raw Frozen Scallop	1. Homogenize (30 s) <b>25 g</b> sample with <b>50 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 2. <b>For water bath:</b> Incubate at <b>39 ± 0.5°C for 7 h.</b> <b>For incubator:</b> Incubate at <b>39 ± 0.5°C for 18 h.</b>	✓ By Actero™ Salmonella Culture Method
Sprout	1. Homogenize (60 s) <b>25 g</b> sample with <b>150 mL pre-warmed (39°C) Actero™ Salmonella Enrichment Media.</b> 2. Incubate at <b>39 ± 0.5°C for 7 h</b> in a water bath. 3. Transfer <b>1,0 mL</b> of enriched sample into <b>10 mL TBG</b> and <b>0,1 mL</b> into <b>10 mL RVS</b> and incubate respectively at <b>43 ± 0.2°C</b> C. and at <b>42 ± 0.2°C</b> for <b>18 h</b> in a water bath.	✓ By Actero™ Salmonella Culture Method

Sample Type	1. Sample Preparation	✓ Analysis of Enriched Samples
Milk Chocolate	2. Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. 3. Incubate at 35 ± 2°C for 22-26 h.	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
	1. Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. 2. Incubate at 39 ± 0.5°C for 18 h.	✓ By Actero™ Salmonella Culture Method
Chocolate Liquor	1. Homogenize (60 s) 25 g sample with 225 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 26-30 h.	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Cocoa Powder	1. Homogenize (60 s) 25 g sample with 175 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media supplemented with 5% NFDLM. 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
Dry Pet Food	1. Homogenize (60 s) 25 g sample with 225 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 18-22 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 2625 mL pre-warmed (35°C) Actero™ Salmonella Enrichment Media. 2. Incubate at 35 ± 2°C for 18-22 h.	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Raw Almond	1. Homogenize (60 s) 375 g sample with 750 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
Peanut Butter	1. Homogenize (60 s) 25 g sample with 175 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
Dried Parsley	1. 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. 2. Incubate at 35 ± 2°C for 20-24 h.	✓ By BAX® System Real-Time PCR Assay for <i>Salmonella</i> ✓ By Actero™ Salmonella Culture Method
Dried Raisin	1. Homogenize (60 s) 25 g sample with 75 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
Whole Black Pepper	1. Homogenize 25 ± 1 g sample manually with 75 ± 5 mL of pre-warmed 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

## Enrichment of Environmental Surface Samples Using Actero™ Salmonella/STEC 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.

### 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/STEC Enrichment Media

### Raw Ground Chicken (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 or mix vigorously in the Stomacher® bag for 1 minute if there is no Stomacher® machine available.
3. Close the 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 ± 0.1 mL to a tube. Cap the tube.

#### 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 35 ± 2°C before use.
2. Add 225 ± 5 mL of pre-warmed Actero™ Salmonella broth to each filter bag containing the 25 ± 1 g test portion.
3. Mix each sample by hand to homogenize it.
4. 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

1. Pre-warm the Actero™ Salmonella broth supplemented with 20 mg/L malachite green at 35 ± 2°C before use.
2. Add 30 ± 1 mL of pre-warmed Actero™ Salmonella broth to each filter bag containing the 30 ± 1 mL test portion.
3. Mix each sample by hand to homogenize it.
4. 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

1. 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  $4 \times 10^5$  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 39-42 ± 0.5°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  $7.0 \pm 0.2$ .
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  $7.0 \pm 0.4$ .
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  $7.0 \pm 0.4$ .
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  $7.0 \pm 0.2$ .
4. Close the bag loosely and incubate the sample upright for **18-22 h at 35°C** in an **incubator** for enrichment.

### Dried Whole Egg (100 g)

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

1. Pre-warm the Actero™ Salmonella broth supplemented with 5% NFDM at **35 ± 2°C** before use.
2. Add **600 ± 10 mL** of pre-warmed Actero™ Salmonella broth supplemented with 5% NFDM to each filter bag containing the **100 ± 5 g** test portion.
3. Mix each sample by hand to homogenize it.
4. Close the bag loosely, and incubate the sample for **14 - 18 h at 35 ± 2°C** using an incubator.

### 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 a 18 h Enrichment in Incubator

1. Close bag loosely and incubate 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.
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

#### 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 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.

#### 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

#### 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. 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.

### Cocoa Powder

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

1. Add **175 ± 5 mL** of pre-warmed (**35 ± 2°C**) Actero™ Salmonella broth supplemented with 5% NFDM to each filter bag containing the **25 ± 1 g** test portion.

2. Homogenize each sample for **1 min** in a Stomacher® 400 circulator or equivalent.
3. Close the bag loosely, and incubate the sample for **16-20 h at 35 ± 2°C** using an incubator.

### Raw Almond

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

1. Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
2. Add **750 ± 25 mL** of pre-warmed Actero™ Salmonella broth to each filter bag containing the **375 ± 10 g** sample.
3. Homogenize each sample for **60 s** using a stomacher.
4. 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

1. Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
2. Add **175 ± 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 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

1. Pre-warm the Actero™ Salmonella broth at **35 ± 2°C** before use.
2. Add **225 ± 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 and then mix manually to remove the dried parsley from the sides of the bags.
4. 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

1. Pre-warm the Actero™ Salmonella broth at **35 ± 2°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 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

1. Pre-warm the Actero™ Salmonella broth at **35 ± 2°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 manually.
4. 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 (39.5°), dried whole eggs, whole liquid eggs:

- ✓ Streak the samples directly 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<sup>®</sup>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 CHROMA<sup>®</sup>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<sup>®</sup>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<sup>®</sup> 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<sup>®</sup> System Real-Time PCR Assay for *Salmonella* for the following steps.

#### **PRODUCT STORAGE AND SHELF LIFE:**

The dehydrated medium and supplement number 1 should be stored at room temperature (12–25°C), in a tightly closed bottle in a cool dry place. Supplement number 2 should be stored at refrigerated temperature (2–8°C) in a cool dry place protected from light. The expiration dates are indicated on the packaging.



The prepared autoclaved medium **without** supplement can be stored for up to 6 months and the **supplemented** medium can be stored for 2 months at 2–8°C and 1 month at 18–25°C, in a tightly closed bottle protected from light. **Please take into consideration that the medium should be autoclaved and manipulated in aseptic conditions.**

#### **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<sup>™</sup> *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-010 (BBFCM-010): Actero<sup>™</sup> *Salmonella*/STEC Enrichment Media, 500 g

#### **FOR FURTHER INFORMATION, PLEASE CONTACT:**

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