



Actero™ Salmonella/STEC Enrichment Media

Product Information

Intended Use:

Actero™ Salmonella/STEC Enrichment Media is used for the selective enrichment of *Salmonella* spp. in liquid whole eggs, raw frozen scallops, sprouts and raw ground chicken and ground beef and on environmental surfaces as stainless steel, plastic, rubber, ceramic tiles and sealed concrete. Furthermore, the medium allow the enrichment of the Shiga toxin-producing *E. coli* (STEC) along or co-enriched with *Salmonella* in ground beef.

The medium has been validated according to the Performance Tested MethodsSM of the AOAC-RI in the above mentioned matrices.

Principle of Operation:

The principle of the patented Actero™ Salmonella/STEC Enrichment Media is based on the unique ability of *Salmonella* and STEC strains to metabolized specific compounds to obtain nutrients essential for bacterial growth. This unique media 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 number 1, 2 bottles of 35 mL.

Actero™ Salmonella/STEC Supplement number 2, 1 bottle of 17 mL.

Additional Materials Required:

1. Distilled/deionized, sterile water.
2. Filtered sterile stomacher bags.
3. Serological pipette, sterile.



4. Non-bactericidal sterile cellulose sampling sponges (8×4×0.3 cm) pre-moistened with neutralizing Dey-Engley buffer. (D/E). (FoodChex Cat # FCSM-003).
5. Water bath 39-40 ± 0.5°C
6. Incubator 39 ± 0.5°C
7. Tips and Adjustable Volume Pipette (100 - 1000 µL).
8. 10 µL calibrated inoculating loop
9. Rappaport-Vassiliadis Broth (RV).
10. Tetrathionate broth.
11. Xylose Lysine Tergitol-4 Agar (XLT4)
12. BG Sulfa Agar (BGS)
13. Xylose Lysine Deoxycholate agar (XLD)
14. Hektoen Enteric agar (HE)
15. Stomacher 3500/Stomacher 400 (Optional) available from multiple sources.
16. Other regular laboratory equipment could also be required.

Procedure: choice of 2 methods for media preparation

Actero™ Salmonella/STEC Enrichment Media Preparation

With the use of AUTOCLAVE

1. Always shake the 500 g dry powder media container before each use.
2. Measure 14.2 grams of dry media powder on the weight scale.
3. Suspend and mix this 14,2 g of the media into a clean one liter bottle of distilled water.
4. Sterilize this bottle of media mixture 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 media must be warmed to **39°C** followed by the **addition of measured amounts from the vials of supplement number 1 and supplement number 2** that have been supplied (see below for amounts to add).
7. Adjust pH to 8.2 ± 0.2 prior to use.

Without the use of AUTOCLAVE



1. Always shake the 500 g dry powder media container before each use.
2. Measure 14.2 grams of dry media powder on the weight scale.
3. Suspend and mix this 14.2 g in one liter of **sterile** distilled water pre-warmed to 39°C.
4. The media equilibrated to 39°C now requires the addition of measured amounts from the vials of supplement number 1 and supplement number 2 that have been supplied (see below for amounts to add).
5. The media prepared should be used **immediately**.
6. Adjust pH to 8.2 ± 0.2 prior to use.

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.

Sample Preparation for 25 g of Ground Chicken

1. Immediately prior to enrichment – add **100 µL** of supplement number 1 and **25 µL** of supplement number 2 to **50 mL of media**. Mix thoroughly by swirling and inverting.
2. Add **50 mL** of pre-warmed **39°C media** to **25 g** of sample in a filter-equipped stomacher bag.
3. Stomach the sample for **30 seconds at 265 rpm** in a Stomacher® 400 circulator or mix vigorously in the stomacher bag for 1 minute if there is no stomacher machine available.
4. Close bag loosely and incubate the samples upright for **20 h at 39°C** in an **incubator** for enrichment.
5. After 20 hours remove the samples from the incubator, re-suspend the contents by shaking the bag and transfer **10.0 ± 0.1 mL** to a tube. Cap the tube.

Salmonella detection

Streak the samples on selective agar plates (XLT4 and BGS) using a calibrated loop of 10 µL and follow the confirmation procedure if necessary as recommended in the USDA FSIS Microbiology Laboratory Guidebook Chapter 4.06.

Sample Preparation for 325 g of Ground Beef

1. Immediately prior to enrichment – add **1.3 mL** of supplement number 1 and **325 µL** of supplement number 2 to **650 mL of pre-warmed 39°C media**. Mix thoroughly by swirling and inverting.



2. Add **650 mL** of pre-warmed media to **325 g** of sample in a filter-equipped stomacher bag.
3. Stomach the sample for **30 seconds at 150 rpm** in a Stomacher® 3500 or alternatively mix vigorously in bag for 1 minute if no stomacher machine available.
4. 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.
5. 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.
6. *Salmonella* detection:
 - a. 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.
 - b. Using a 10 µL inoculating loop, streak to BGS and XLT4 and incubate at 35°C for 18-24 h.
 - c. Follow the confirmation procedure if necessary as recommended in the USDA FSIS Microbiology Laboratory Guidebook Chapter 4.06.
7. STEC detection:
 - a. After the enrichment, follow the procedure as recommended in the USDA FSIS Microbiology Laboratory Guidebook Chapter 5B.03.

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

Sample Preparation for 100 g Liquid whole Eggs

For a 7 h Enrichment in Water Bath

1. Immediately prior to enrichment – add **600 µL** of supplement number 1 and **150 µL** of supplement number 2 to **300 mL of pre-warmed 39°C media**. Mix thoroughly by swirling and inverting.



2. Add this **300 ml** of pre-warmed 39°C media to **100 g** of liquid whole eggs in a filter-equipped stomacher bag. Adjust pH, if necessary, to 7.0 ± 0.4 .
3. Stomach the sample for 30 seconds at 150 rpm in a Stomacher[®] 3500 or alternatively mix vigorously in bag for 1 minute if no stomacher machine available.
4. Close bag loosely and incubate the sample upright for **7 hours 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.
5. 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. Immediately prior to enrichment – add **1.4 mL** of supplement number 1 and **350 µL** of supplement number 2 to **700 mL** of pre-warmed 39°C media. Mix thoroughly by swirling and inverting.
2. Add this **700 ml** of pre-warmed 39°C media to **100 g** of liquid whole eggs in a filter-equipped stomacher bag. Adjust pH, if necessary, to 7.0 ± 0.4 .
3. Stomach the sample for **30 seconds at 150 rpm** in a Stomacher[®] 3500 or alternatively mix vigorously in bag for 1 minute if no stomacher machine available.
4. Close bag loosely and incubate the sample upright for **18 hours at 39°C in an incubator** for enrichment.
5. 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.

Salmonella detection

Streak the samples on selective agar plates (XLT4 and BGS) using a calibrated loop of 10 µL and follow the confirmation procedure if necessary as recommended in the USDA FSIS Microbiology Laboratory Guidebook Chapter 4.06.



Sample Preparation for 25 g of Raw Frozen scallops

1. Immediately prior to enrichment – add **100 µL** of supplement number 1 and 25 µL of supplement number 2 to **50 mL of pre-warmed 39°C media**. Mix thoroughly by swirling and inverting.
2. Add **50 ml** of pre-warmed media to **25 g** of raw frozen scallop sample in a filter-equipped stomacher bag.
3. Stomach sample for **30 sec. at 265 rpm** in a Stomacher® 400 circulator or alternatively mix vigorously in bag for 1 minute if no stomacher machine available.

For a 7 h Enrichment in Water Bath

1. Close bag loosely and incubate sample upright for **7 hours 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 hours 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

Salmonella detection

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



Sample Preparation for 25g of Sprouts

1. Immediately prior to enrichment – add **300 µL** of supplement number 1 and **75 µL** of supplement number 2 to **150 mL of media**. Mix thoroughly by swirling and inverting.
2. Add **150 ml** of pre-warmed 39°C media to **25 g** of sprout sample in a filter-equipped stomacher bag.
3. Stomach sample for **1 min. at 265 rpm** in a Stomacher® 400 circulator or alternatively mix vigorously in bag for 1 minute if no stomacher machine available.
4. Close bag loosely and incubate sample upright for **7 hours 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.
5. 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
6. From the enriched Actero™ Salmonella sample, transfer **1.0 ml 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).

Salmonella detection

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

Environmental Surface Samples (Stainless Steel, Plastic, Rubber, Ceramic Tile and Sealed Concrete)

1. Add to the non-bactericidal, non-bacteriostatic 8×4×0.3 cm sterile cellulose sampling sponge, the content of a tube of D/E buffer provided with the kit.
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. The sample should be tested within 8 h.
 4. When ready to test, pre-warm the prepared Actero™ Salmonella Enrichment Media at **39 °C**.
 5. Add **90 ± 5 mL** of the pre-warmed Actero™ Salmonella Enrichment Media to each sponge sample in its sample bag.
 6. Stomach the sample for **30 seconds at 265 rpm** in a Stomacher® 400. Hand mixing, is an acceptable alternative for stomaching. To hand mix, massage each sponge that is in the sealed stomacher bag for approximately one minute.
 7. For the enrichment phase, close the bags and incubate the samples in an incubator for **18 ± 0.5 h** at **39 ± 0.5 °C**. Adherence to temperature is important for accurate results.
 8. At the end of the enrichment period, mix sample thoroughly and transfer **10.0 ± 0.1 mL** of the enriched sample to a tube. Cap the tube.
 9. Streak the samples on selective agar plates (XLD and HE) using a calibrated loop of 10 µL and follow the confirmation procedure if necessary as recommended in the US FDA Bacteriological Analytical Manual Chapter 5.

Interpretation and Test Result Report

All samples presenting typical colonies after 48h in the selective agar should be consider as presumptive positives. The presumptive results confirmed according the US FDA Bacteriological Analytical Manual Chapter 5 and USDA FSIS Microbiology Laboratory Guidebook Chapters 4.06 and 5B.03.

All samples which do not present typical colonies after 48h of incubation can be consider as negative samples.

Product Storage and Shelf Life:

The dehydrated media and the supplement number 1 should be stored at room temperature (15–25°C), in tightly closed bottle in a cool dry place. The 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 media **without** supplement can be stored for up to 6 months and the **supplemented** media can be stored for 1 month in tightly closed bottle at 2–8°C, in a cool dry place protected from light. **Please take in consideration that the media should be autoclaved and manipulated in aseptic conditions.**

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 and STEC 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[™] STEC/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., STEC 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:

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FCM-010 : ActeroTM Salmonella/STEC Enrichment Media

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