FoodChek™
Food safety, simplified.

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Canada

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Actero™ Salmonella/STEC Enrichment Media

Product Information

Intended Use:

Actero™ Salmonella/E. coli Enrichment Media is used for the selective growth of *Salmonella* and *E. coli* spp. Actero promotes selective fast *Salmonella* spp. growth in food samples of whole liquids eggs, raw frozen scallops, sprouts, raw ground chicken and ground beef. This media will also promote faster selective growth and enrichment of the Shiga toxin-producing *E. coli* (STEC) along with Salmonella in food samples of ground beef. The Actero media inhibits the growth of bacteria that are not either *Salmonella* spp. or *E. coli* spp.

Principle of Operation:

The principle of the patented Actero™ Salmonella/STEC Enrichment Media is based on the ability of *Salmonella* and STEC strains to optimize growth by the use of specific nutrients that are contained within the Actero media and inhibits growth of bacteria that are not *Salmonella* or E. *coli* spp.

Kit Contents:

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

Dehydrated Actero™ Salmonella/STEC Enrichment Media, bottle of 500g.

Actero™ Salmonella/STEC Supplement number 1 (2 bottles of 35mL).

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. Water bath 39°C ± 0.5°C
5. Incubator 39°C ± 0.5°C
6. Tips and Adjustable Volume Pipette (100 - 1000 µL).
7. 10µL calibrated inoculating loop
8. Rappaport-Vassiliadis Broth (RV).
9. Tetrathionate broth.

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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.

15.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 500g 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).

Without the use of **AUTOCLAVE**

1. Always shake the 500g dry powder media container before each use.
2. Measuring 14.2 grams of dry media powder on the weight scale.
3. Suspend and mix the 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 number 2 that have been supplied (see below for amounts to add)
5. The media prepared should be used immediately.

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Sample Preparation for 25g 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 25g of sample in a filter-equipped stomacher bag
3. Stomach the sample for 30 seconds at 265 rpm in a Stomacher® 400 circulator or alternatively 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 twenty (20) hours at 39°C in an incubator for enrichment.
5. After 20 hours remove the sample from the incubator and re-suspend the contents by shaking the bag.
6. Streak the sample onto selective agar plates (XLT4 and BGS) using a calibrated loop of 10 µL. Follow the confirmation procedure if necessary as recommended in the USDA FSIS Microbiology Laboratory Guidebook Chapter 4.05.

Sample Preparation for 325g 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 39°C 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 sample upright for 7 hours 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 in the incubator reaches 39.5°C before starting to record the required incubation time.
5. After 7 hours remove the sample from the water bath and mix the contents by shaking the bag.
6. Dispense 100µL of sample from the bag onto selective agar plate (XLT4 and BGS) and streak using a 10µL inoculating loop to obtain isolated colonies.
7. Follow the confirmation procedure when there is a positive result as recommended in the USDA FSIS Microbiology Laboratory Guidebook Chapter 4.05.

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

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Sample Preparation for 100g Whole Liquid Eggs

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 100g of whole liquid eggs in a filter-equipped stomacher bag. Adjust pH, if necessary, to 7.0.
3. Stomach this sample for 30 seconds at 150 rpm in a Stomacher® 3500. or alternatively mix vigorously in stomacher 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. After 7 hours, remove the sample from the water bath and mix the contents by shaking the bag.
5. Streak the sample 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 4.05.

Sample Preparation for 25g of Raw Frozen Scallops

1. Immediately prior to enrichment – add 100 µL of supplement 1 and 25 µL of supplement 2 to **50 mL of pre-warmed 39°C media**. Mix thoroughly by swirling and inverting.
2. Add 50 ml of pre-warmed 39°C media to 25g 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 stomacher bag for 1 minute if no stomacher machine available.
4. Close bag loosely and incubate samples 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.
5. After 7 hours, remove the sample from the water bath and mix the contents by shaking the bag.
6. 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 United States FDA Bacteriological Analytical Manual Chapter 5

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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 25g 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 required incubation time. After 7 hours, remove the sample from the water bath and mix content by shaking the bag.
5. From the enriched Actero™ Salmonella/STEC sample, transfer 1.0 ml into 10 mL Tetrathionate Broth and incubate at 42 ± 0.2°C for 18 hours. Respectfully and separately transfer 0.1 ml into 10 mL Rappaport-Vassiliadis broth (RV) and incubate at 42 ± 0.2°C for 18 hours.
6. After this enrichment, streak the sample onto separate selective agar plates (XLD and HE) using a calibrated loop of 10 µL. Follow the confirmation procedure when there is a positive result as recommended in the United States 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 4.05 and 5B.01.

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

Product Storage and Shelf Life:

The dehydrated media and the supplement 1 should be stored at room temperature (15–25°C) in a tightly closed bottle in a cool dry place. The supplement 2 should be stored at

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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 a tightly closed bottle at 2–8°C, in a cool dry place protected from light. Please take into consideration that the media should be autoclaved and always manipulated under aseptic conditions.

Disposal:

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

Precautions:

Salmonella are generally categorized as Biosafety Level 2 pathogens. The use of microbiological media such as the FoodChek™-Actero Salmonella/STEC Enrichment Media requires trained laboratory personnel familiar with good microbiological laboratory practices. Wearing a laboratory coat, disposable gloves and eye protection while handling media specimens and performing the assay is strongly recommended. The 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 startup.

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:

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 statue, (implied warranties) are expressly excluded.

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Catalogue Number:

FCM-009 : Actero Salmonella Enrichment Media
FCM-010 : Actero Salmonella/STEC Enrichment Media
FCM-013 : Actero STEC Enrichment Media

For further information please contact:

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