

## Actero™ STEC Enrichment Media Product Information

### INTENDED USE:

Actero™ STEC Enrichment Media is used for the selective growth of *Shiga-Toxin-producing E. Coli* (STEC). Actero™ promotes faster selective growth and enrichment of the STEC strains.

It has been validated according to the Performance Tested Methods<sup>SM</sup> of the AOAC-RI in raw ground beef.

### PRINCIPLE OF OPERATION:

The principle of the patented Actero™ STEC Enrichment Media is based on the ability of STEC strains to optimize growth by the use of specific nutrients that are contained within the Actero™ media. 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™ STEC Enrichment Media, a bottle of 500 g.

Actero™ STEC Supplement number 1, 2 bottles of 35 mL.

Actero™ 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 39-40 ± 0.5°C
5. Incubator 39 ± 0.5°C
6. Tips and Adjustable Volume Pipette (100 - 1000 µL).
7. 10 µL calibrated inoculating loop
8. Stomacher 3500/Stomacher 400 (Optional) available from multiple sources.
9. Other regular laboratory equipment could also be required.

### PROCEDURE:

#### Actero™ 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 of refrigerator 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.

##### 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 the bag for 1 minute if no stomacher machine is available.
4. Close the bag loosely and incubate the samples 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 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. 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 x 10<sup>5</sup> CFU/g

##### Interpretation and Test Result Report

All samples presenting typical colonies after 48h in the selective agar should be considered as presumptive positives. The presumptive results should be confirmed according to the USDA FSIS Microbiology Laboratory Guidebook Chapters 5B.03.

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

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

The dehydrated media 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 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 in consideration that the media 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:**

STEC strains 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™ STEC 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 STEC strains 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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**CATALOGUE NUMBER:**

FCM-013: Actero™ STEC Enrichment Media

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