



<u>FSI Catalogue No.</u>	<u>VWR Catalogue No.</u>	<u>Description</u>
<b>FCM-040</b>	<b>89522-696</b>	<b>Actero™ MediaBox™ modified TSB Broth (5 Liter)</b>
<b>FCM-041</b>	<b>89522-698</b>	<b>Actero™ MediaBox™ modified TSB Broth (10 Liter)</b>
<b>FCM-042</b>	<b>89522-700</b>	<b>Actero™ MediaBox™ modified TSB Broth (20 Liter)</b>

MediaBox modified TSB, specifically designed for the enrichment of *Escherichia coli* O157 from food and can be used with the FoodChek™ VCC supplement (FCM-002) for the selective enrichment of *Escherichia coli* O157 prior to detection with FoodChek™ E. coli Assay Cassettes (FCEC-006)

**INTENDED USE**

MediaBox™ modified TSB broth is a partially selective broth used for the initial enrichment of *E. coli* from foods. **MediaBox™ modified TSB broth is not intended for Clinical or veterinary use.**

**SUMMARY & EXPLANATION**

This medium was developed to facilitate the selective enrichment of *E. coli* O157

**Storage Instructions:** On receipt, store the MediaBox at room temperature 15 - 25°C.

**Composition:**

Casein Digest Peptone..... 17.0g  
 Papaic Digest of Soybean Meal..... 3.0g  
 Sodium Chloride.....5.0g  
 Dextrose.....2.5g  
 Dipotassium Phosphate.....4.0g  
 Bile Salts No. 3.....1.5g

**Final pH:** 7.3 ± 0.2 at 25°C

**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 intend to use to control dispensing of the broth.  
 Have your sterile connecting tubing with 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.

To enrich *E. coli* O157 from a food sample, consult FoodChek E. coli O157 package insert, FDA:BAM, or any other appropriate reference.

**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.

**PROCEDURE FOR USING MEDIABOX™ MTSB FOR THE DETECTION OF E.coli O157 USING THE FCEC-006 ASSAY CASSETTES:**

**Additional Materials Required:**

1. Media Supplement – The media supplement provides additional selectivity to the enrichment broth to ensure growth of the target to the detection threshold. The media supplement is a lyophilized powder in a septum vial. The user hydrates the supplement with 20 mL of sterile distilled water before addition to the enrichment broth. Two vials of supplement media are included in the kit. If additional media supplement is required, it is available from FoodChek Systems Inc. catalogue number FCM-002.





2. Small volume polypropylene or reusable glass tube with cap.
  3. Serological pipette, sterile.
  4. Stomacher bag with filter.
  5. Distilled/deionized, sterile water.
  6. Water bath at 39°C +/-1°C.
  7. Tips and Adjustable Volume Pipette (100 - 1000 µL).
  8. Stomacher (optional). Available from multiple sources such as Seward, Fisher Scientific.
  9. FoodChek™ Reader – available from FoodChek Systems Inc, catalogue number FCR-004.
  10. SANI-SPONGE kit (cat. number KSS-61130-BP, LabPlas Inc., St-Julie, Quebec, Canada). This material is required **only** for carcass sponge protocol.
4. Add two parts of pre-warmed medium (containing supplement) to one part of sample (Ex. 750mL of supplemented medium to 375g of ground beef) in a filter-equipped stomacher bag.
  5. Stomach the sample for 30 seconds at 150 rpm in a Stomacher® 3500.
  6. Close bag loosely and incubate the samples for 7 hours at 39°C in a water bath for enrichment. If a large number of samples is to be analyzed, verify that the temperature of the water 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 accurate results.
  7. After 7 hours remove the samples from the water bath and re-suspend the contents.

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 25g, 65g; 375g or a 325g composited ground beef samples (Sensitivity 1cfu/375g of ground beef).**

1. Warm medium base to 39°C. Note: for optimum assay performance it is critical that the medium is equilibrated to 39°C before use. This is most easily done by holding the medium overnight (10-20 hours) in an incubator or for few hours in a water bath.
2. Reconstitute a vial of lyophilized media supplement (**FCM-002**) by adding 20 mL of sterile water and mix. Store unused liquid portions refrigerated for up to 10 days.
3. Immediately prior to enrichment add 2.2 mL (±0.1 mL) of media supplement to 1L of pre-warmed medium base. Mix thoroughly by swirling and inverting.

**Sample Preparation for 375g composited beef trims samples (Sensitivity 1cfu/375g of beef trims).**

1. Warm media base to 39°C. Note: for optimum assay performance it is critical that the media is equilibrated to 39°C before use. This is most easily done by holding the media overnight (10-20 hours) in an incubator or for few hours in a water bath.
2. Reconstitute a vial of lyophilized media supplement (**FCM-002**) by adding 20 mL of sterile water and mix. Store unused liquid portions refrigerated for up to 10 days.
3. Immediately prior to enrichment add 2.2 mL (±0.1 mL) of media supplement to 1L of pre-warmed media base. Mix thoroughly by swirling and inverting.
4. Add 750 ml of pre-warmed media (containing supplement) to 375g of beef trims in a filter-equipped stomacher bag.
5. Stomach the sample for 45 seconds at 175 rpm in a Stomacher® 3500.
6. Close bag loosely and incubate the samples for 6 hours at 39°C in a water bath for enrichment. If a large number of samples is to be analyzed, verify that the temperature of the water 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 accurate results.

7. After 6 hours, remove the samples from the water bath and re-suspend the contents.

Note: This test was standardized to be used with meat having a maximum account of aerobic total of  $3 \times 10^6$  cfu/g

**Sample Preparation for carcass sponge (Sensitivity < 0.05 cfu/100 cm<sup>2</sup> on post-wash beef carcass).**

1. Warm media base to 39°C. Note: for optimum assay performance it is critical that the media is equilibrated to 39°C before use. This is most easily done by holding media overnight (10-20 hours) in an incubator or for one hour in a water bath.
2. Reconstitute a vial of lyophilized media supplement (**FCM-002**) by adding 20 ml of sterile water and mix. Store unused liquid portions refrigerated for up to 10 days.
3. Immediately prior to enrichment – add 133 µl ( $\pm 0.5 \mu\text{l}$ ) of media supplement to 60 ml of pre-warmed media base. Mix thoroughly by swirling and inverting.
4. Add 60 ml of pre-warmed media (containing supplement) to each carcass sponge sample in a stomacher bag.
5. Stomach sample for 30 sec. at 265 rpm in a Stomacher® 400 circulator.
6. Close bag loosely and incubate samples for 6 hours at 39°C in a water bath for enrichment. If a large number of samples is 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.
7. After 6 hours, remove the samples from the water bath and mix content.

**NOTE:** the test was standardized using SANI-SPONGE kit (LabPlas Inc., St-Julie, Quebec, Canada). We strongly suggest using this kit or another cellulose sponge 1.8 x 3.2 in, pre-moistened with 10 ml of the Butterfield phosphate buffer-sterile. Sponge should be wrung out in a Sterile Sampling bag before swabbing.

**General Preparation**

1. Bring the required number of cassettes to ambient temperature at least 10 minutes prior to use.
2. Turn on the FoodChek™ reader. Initialization will take a few minutes. When instrument is ready for use, display will show four pictograms. Touch the pictogram which represents a cassette.

**Analysis**

1. Place one identified tube (with the cap removed) for each sample into the rack.
2. Mix sample thoroughly and transfer 10.0 ml ( $\pm 0.2$  mL) of the enriched samples to the tubes. Cap the tubes.
3. Heat the samples in boiling water for 15 minutes.
4. After heating, let the samples cool to ambient temperature.
5. Mix each sample thoroughly.
6. Using a micropipette, transfer 200 µL of heat-treated sample (cooled) directly to the sample port of a FoodChek™-E. coli O157 cassette at ambient temperature. Change pipette tips between samples.
7. Allow the assays to develop for 30 minutes at ambient temperature before reading in the FoodChek™ reader. Note: interpreting results before 25 minutes or after 35 minutes may yield inaccurate results.
8. Open the door on the front of the reader and insert the cassette.
9. Closing the reader door initiates the reading process and generates an output on the LCD screen and a printed result on paper tape.

**Interpretation and Test Result Report**

Results are reported on both the instrument LCD display and the instrument printout. Result is indicated as either "Positive (+)" or "Negative (-)".

Since FoodChek™-E. coli is approved as a screening test for E. coli O157 all positive samples should be





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culturally confirmed by an approved USDA/FSIS or HC/CFIA confirmatory method.

**RESULTS mTSB**

After initial enrichment of samples spiked with *E. coli* O157 or another STEC *E. coli* at low concentrations (less than 10 cfu) for 24 hours at 35°C ± 2°C streak 10-20µl of the sample onto a suitable chromogenic agar plate. The plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

For the isolation of *E. coli* from food, food ingredients, air, water or other materials consult FoodChek *E. coli* 0157 package insert, FDA:BAM or any other appropriate reference.

**LIMITATIONS OF THE PROCEDURE**

This ready to use broth is intended for primary enrichment. Further selective enrichment in a selective broth and or on a selective agar plate would be required to isolate a pure culture.

