



FoodChek™-E. coli O157 Kit for the Detection of *E. coli* O157

Product Information

Intended Use:

The FoodChek™-E. coli O157 kit is a rapid detection assay for *Escherichia coli* O157 in raw ground beef, beef trims and carcass sponge. The test kit permits presumptive detection of *Escherichia coli* O157 (including H7) when present at low levels (please, see below the sensitivity for each matrix). The system is comprised of a magnetic nanoparticle immunoassay (the FoodChek™ cassette), an automated magnetic reader (the FoodChek™ reader) and a commercially available enrichment media. This assay provides rapid results, and compared to most other methods, requires less than eight (8) hours for a total test time.

FoodChek™-E. coli O157 has been certified for use with raw ground beef, beef trims and carcass sponge by AOAC *Performance Tested Methods*SM, License Number 060902.

Principle of Operation:

The kit is based on the use of antibody-coated super-paramagnetic nanoparticles in a lateral flow immunoassay format. Unlike typical lateral flow assays it does not depend on a visual inspection of the assay cassette to determine if the result is positive or negative – a procedure that is highly subjective at low concentrations of analyte. Instead the cassette is read in a magnetic detection system (the FoodChek™ reader), which confirms the presence of *Escherichia coli* O157.

Apart from the FoodChek™ reader the kit requires only standard laboratory equipment and supplies. A supplemented commercially available growth media for enriching the sample is also required.

Kit Contents:

The kit contains twenty FoodChek™-E. coli O157 cassettes and two vials of the media supplement. The cassette is a lateral flow immunochromatographic device. The cassette carries a printed label that contains written and bar-coded information on the identity of the cassette, the expiration date and the required values for automated analysis by the FoodChek™ reader. The cassette cannot be visually interpreted and requires the use of the FoodChek™ reader.

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.



Additional Materials Required:

1. Enrichment media – The broth is prepared from dehydrated powder by re-suspension of 33g per liter of distilled water, then autoclaved. Alternatively the media powder could be mixed directly with sterile water for immediate use. It is used in conjunction with a media supplement (see above). The recommended commercial media is available from FoodChek Systems Inc. catalogue number FCM-001 for the 500g bottle format and FCM-003 for the pouch format.
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.

Procedure:

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 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.



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.

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 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 3×10^6 cfu/g



Sample Preparation for carcass sponge (Sensitivity < 0.05 cfu/100 cm² 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 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 (±0.5µl) 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 (±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 culturally confirmed by an approved USDA/FSIS or HC/CFIA confirmatory method or send to Laboratoires de diagnostic moléculaire et virologie, Faculté de médecine vétérinaire, Université de Montréal, 3200 rue Sicotte, Saint-Hyacinthe, Qc, J2S 7C6, Tél.: 450-773-8521 ext. 8375, Fax: 450-778-8108 to be confirmed by a Real time PCR specific for *E. coli* O157.

Product Storage and Shelf Life:

Store the product at 2°C – 8°C until ready for use. The shelf life of the kit is 18 months from date of manufacture, as marked on the cassette.

Disposal:

Dispose of all used cassettes, pipettes and 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:

Microbiological tests such as FoodChek™-E. coli require 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.

All enrichment broths may contain various pathogens whether they contain *E. coli* O157:H7 or not. Furthermore, *E. coli* O157 has a very low infective dose (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 statute, (implied warranties) are expressly excluded.

FoodChek Systems Inc. warrants its new equipment to the original Customer only for a period of one (1) year after date of delivery against defects in material and workmanship and defects arising from failure to conform to FoodChek Systems Inc.'s specifications applicable on the date of delivery. FoodChek Systems Inc.'s sole obligation under this warranty shall be to replace or repair the defective product or part, for any defect found to have occurred under normal use during the one (1) year period. This warranty does not cover replacement of products damaged due to misuse, abuse, alteration, self repair, loss or theft.

Catalogue Number:

FCEC-001: FoodChek™-E. coli O157 Kit for the Detection of *E. coli* O157

For further information please contact:

FoodChek Systems, Suite 450, 1414 8th Street S.W. Calgary, Alberta, Canada T2R 1J6. Tel: +1 (403) 269-9424 or your FoodChek™ distributor.





****IMPORTANT NOTICE****

CULTURAL CONFIRMATION OF FOODCHEK™ - E. COLI O157 POSITIVE RESULT

If any positive result in a sample is detected while using the FoodChek™ - E. coli O157 assay cassette, it is recommended to use the following process for cultural confirmation.

Instructions for the institution or processor that found the initial positive result:

1. All presumptive positive samples detected by FoodChek™ - E. coli O157 test system should be considered as potential positives. Start isolation procedures from the enrichment culture in the stomacher bag.
2. For each sample that you want culturally confirmed, place one labeled tube into a rack.
3. Mix sample thoroughly and transfer 5.0mL (± 1 mL) of the enriched sample into the tube.
4. Ship the samples to the external laboratory in a cooler with ice packs. The shipping should be done according to local, municipal, provincial, state and/or federal regulations concerning the transport of biohazard material.

Ensure the following Instructions are given to the external laboratory:

5. Upon receipt of the sample, aliquot 1ml of the sample into 9mL of mTSB+n (modified TSB + novobiocin at final concentration of 20mg/L) and mix thoroughly.
6. Incubate the mix for 18 to 22 hours at $42 \pm 1^\circ\text{C}$.
7. After incubation, follow the MLG 5.06 method starting at section 5.6 described in the Microbiology Laboratory Guidebook of the FSIS.

****Note** Steps 1 to 5 should be done within 24 hrs.**