

# SOLUS ONE

## *E. coli* O157

Immunoassay-Based Test System for the  
Detection of *E. coli* O157  
in Selected Food Samples

**Insert Number:** 34  
**Issue Number:** 2.0  
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**Product Code(s):** EC1-0096, EC1-0480  
**Certifying Body:** AOAC





This method's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

This method has been evaluated in the AOAC® *Performance Tested Methods*<sup>SM</sup> Program for the detection of *E. coli* O157 in raw ground beef and raw beef trim. Solus One *E. coli* O157 method has been compared to the USDA-FSIS MLG 5C.00 reference method. (AOAC® *Performance Tested*<sup>SM</sup> License no. **112001**).

## 1. INTRODUCTION

Solus One *E. coli* O157 provides a negative or a presumptive positive result from a single enrichment step within 14 hours, including the assay time.

## 2. INTENDED USE

Solus One *E. coli* O157 is for the same day detection of *E. coli* O157, including H7, in selected food samples. The test method is easy to perform, however it requires laboratory facilities plus qualified and trained personnel. Basic training is recommended to first time users and is given by Solus Scientific Solutions Ltd.

Using the method includes compliance with Good Laboratory Practices (refer to EN ISO 7218).

## 3. REAGENTS PROVIDED

Most kit components are supplied stabilized and ready to use at working concentration with only the Washing Buffer concentrate requiring dilution. Each kit contains sufficient material for 1 (EC1-0096) or 5 (EC1-0480) x 93 determinations, plus controls. The kit expiry date is displayed on each product label.

Component	Appearance	Volume		Comments
		EC1-0096	EC1-0480	
Assay plate	96-well microplate with removable/ breakable strip format	1	5	Wells coated with antibodies against <i>E. coli</i> O157.
Negative control	Colorless liquid. Green label.	3ml	10ml	Working concentration. Contains diluent with preservative.
Positive control	Black liquid. Red label.	3ml	10ml	Working concentration. Contains heat-killed <i>E. coli</i> O157 in diluent with preservative.
Conjugate	Colorless/very pale straw-colored liquid. Orange label.	11ml	60ml	Working concentration. Contains horseradish peroxidase antibody conjugate in diluent with preservative.
Substrate	Colorless/very pale blue liquid. Blue label.	11ml	60ml	Working concentration. Contains 3, 3', 5, 5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilizers.
Stop solution	Colorless liquid. Silver label.	11ml	60ml	Working concentration. Contains 0.2M sulfuric acid.
Washing buffer concentrate	Colorless/yellow/orange liquid. White label.	10ml x 6	60ml x 5	Concentrated. Dilute before use.
Frit filters	Flat, white plastic filter discs	100	500	Frit filters should be applied to each sample tube after heat-treatment once the sample has cooled back to ambient temperature (18-25°C). See section 7 for details.

## 4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Refrigerator at 2-8°C	Vortex mixer
Deionized or distilled (DI) water	Timer
Buffered Peptone Water (BPW) pre-warmed to 41.5°C	Incubator at 37±1°C
Solus One Supplement	Incubator at 41.5±1°C
70% v:v Ethanol	Tubes for sample boiling (e.g. 5ml polypropylene rimless tubes 12x75mm)
Filter bags	
Homogenizer (or similar apparatus)	Heating apparatus (e.g. heat block) capable of heating to 85-100°C
Measuring cylinders for various volumes (e.g. 250 ml, 1L)	
Pipettes and tips (1ml; 0.1ml)	Dynex DS2 or Microplate washer and microplate reader with 450nm filter
3ml transfer pipettes (sterile)	
	Autoclave for decontamination of samples

## 5. REAGENT PREPARATION

### 5.1 Wash Buffer:

Prepare the following in a clean vessel.

EC1-0096	EC1-0480
Add 10ml (1 bottle) of the washing buffer concentrate to 240ml DI water and swirl to mix.	Add 60ml (1 bottle) of the washing buffer concentrate to 1440ml DI water and swirl to mix.

### 5.2 Culture Broth (growth medium):

- Prepare Buffered Peptone Water (BPW) following manufacturer's instructions. Allow to cool to ambient temperature (e.g. 18-25°C) before use.
- Reconstitute the Solus One Supplement according to the manufacturer's instructions. Prepare supplemented culture broth (BPW) at the level appropriate to the supplement pack size being used and the strength required for the matrix to be tested. See section 6 for details.

Matrix	Supplement concentration	SALSUPP22.5 or SALSUPP112.5	SALSUPP200
Raw ground beef, raw beef trim	"Half strength"	Add 2.22ml of supplement per 1L of culture broth	Add 1.25ml of supplement per 1L of culture broth

NOTE: Ensure BPW is pre-warmed prior to addition of Solus One Supplement where required as per section 6.

## 6. SAMPLE PREPARATION AND ENRICHMENT

### 6.1 Food Samples

#### 1 in 4 dilution in half strength supplemented BPW:

- 375g sample (Raw ground beef, raw beef trim) - Warm BPW to 41.5±1°C prior to supplementing at half strength for use. Homogenize 375g of the sample in 1125ml of pre-warmed & supplemented BPW and incubate for 10-12 hours at 41.5±1°C.

Some enriched sample types can contain particulates that make pipetting difficult. Use of a filter bag for enrichment can contain these particulates and minimize pipetting issues.

Ensure that the bench processing time of samples is kept to a minimum and that transfer to 41.5°C incubator occurs as soon as possible. This is important to avoid extensive growth of competing organisms.



## 7. POST ENRICHMENT HEAT INACTIVATION

- 7.1. When the sample incubation period is completed, transfer 1-2ml aliquot (avoiding particulates) to a sample boiling tube (e.g. 5ml polypropylene tube).
- 7.2. Heat the aliquot to 85-100°C for 15-20 minutes in the tube. After heating, allow the sample to cool to ambient temperature (18-25°C). This may be accelerated by placing the tubes in cold tap water for ~5 minutes.
- 7.3. To prevent pipetting issues, especially on the Dynex DS2 instrument, add a frit filter directly into each sample tube and gently push down to allow pipetting of a relatively clear sample from above the level of the frit.

NOTE - The frit should only be inserted after the heating stage, and once the tube has cooled back to ambient temperature (18-25°C).

The non-heat-inactivated samples should be kept for verification until immunoassay results are obtained. These samples should be kept at 41.5±1°C if the test is to be carried out within 2 hours. If this is not possible, keep the broths for up to 72 hours at 2-8°C prior to the test.

## 8. IMMUNOASSAY PROCEDURE

- 8.1. Take test kit from storage at least one hour before use to allow the components to reach ambient temperature (18-25°C). Determine the number of wells required for the test. Take required number of strips from the pouch and fit them to the frame provided. Unused strips should be returned to the pouch and stored at 2-8°C.
- 8.2. Prepare Wash Buffer as detailed in section 5.1 for the kit size being used.
- 8.3. Leave the first well in the strip empty to serve as a 'blank' for measuring the absorbance of the substrate.
- 8.4. Pipette 0.1ml of Negative Control (Green label) into the second well.
- 8.5. Pipette 0.1ml of Positive Control (Red label) into the third well.
- 8.6. Pipette 0.1ml of each heat-inactivated sample separately into consecutive wells in the strip. If there are wells left over at the end of a test strip the Positive or Negative Controls may be repeated. †
- 8.7. Incubate the plate (containing the strips) at 37±1°C for 30-35 minutes.
- 8.8. After incubation, aspirate the contents of the wells, removing as much of the liquid as possible. Wash the wells 5-7 times with wash buffer ensuring complete filling and emptying of the wells through each wash cycle. The washing technique is critical to assay performance, hence it is recommended to use a microplate washer instrument.
- 8.9. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.
- 8.10. Incubate the plate at 37±1°C for 30-35 minutes.
- 8.11. Repeat the wash cycles as detailed in section 8.8
- 8.12. Pipette 0.1ml of Substrate (Blue label) into all wells, including the 'blank' well.
- 8.13. Incubate the plate at ambient temperature (18-25°C) for 30-35 minutes in the dark.
- 8.14. After incubation, stop the reaction by adding 0.1ml of Stop Solution (Silver label) to all wells including the 'blank' well. The stop solution will cause any blue color in wells to change to yellow.

8.15. Read the optical densities of wells within 10 minutes in a plate reader using a 450nm filter. Before reading, inspect the wells for air bubbles and, if present, burst with a needle. The reader should be zeroed against the 'blank' well before the other wells are read. Do not use reference filter. The use of automatic ELISA equipment is preferred and should be set up and validated to this protocol.

† If using the Dynex instrumentation, care must be taken to avoid bubbles in the sample and reagent tubes, or films forming across the tube above the level of the liquid. It is essential to check that the system has successfully pipetted samples into the assay plate before proceeding.

## 9. INTERPRETATION OF RESULTS

Results are expressed as optical density ( $OD_{450}$ ) measurements using a microplate reader.

Acceptance criteria:

Negative Control $OD_{450}$	< 0.100
Positive Control $OD_{450}$	> 0.500

The value of the blanking well (usually A1 when processing manually) should always be subtracted from all other results. Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be performed again.

Samples with  $OD_{450}$  readings of < 0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding non-heat-inactivated samples may be discarded following local regulations/guidelines.

Sample with  $OD_{450} \geq 0.200$  are considered presumptive positive for *E. coli* O157. Presumptive positive results must be verified using a recognized culture method.

## 10. CONFIRMATION OF POSITIVE RESULTS FROM ONE *E. COLI* O157 IMMUNOASSAY

All samples identified as positive must be confirmed using the conventional tests described in the standardized methods by the FDA (BAM Chapter 4A) or USDA (MLG 5C). The confirmation step must start from the non-heat-inactivated samples stored during section 7.

## 11. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. DO NOT FREEZE. The kit expiry date is displayed on the kit box plus all of the kit components within the box. Any unused diluted wash buffer can be stored for up to 10 days if kept at 2-8°C. Any unused microplate strips should be returned to the foil pouch with the desiccant sachet and the seal closed completely, then stored at 2-8°C.

## 12. SAFETY

While the procedures detailed are simple and easy to perform, they require laboratory facilities with qualified personnel trained for the handling of potentially pathogenic organisms. Training is recommended to first time users and is provided by Solus Scientific Solutions Ltd.

- The Stop Solution contains sulfuric acid which is corrosive. Wash immediately with large quantities of water if the solution comes into contact with skin or mucous membranes.



As a guide, the following precautions should be taken as a minimum:

- Protective clothing should be worn including lab coat, safety glasses, mask and gloves where appropriate.
- Do not pipette by mouth.
- Avoid contact with the skin.
- Do not eat, drink or apply cosmetics in the laboratory.
- Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes.

### 13. PRECAUTIONS FOR OPTIMAL PERFORMANCE

- Reagents are provided at fixed working concentration. Optimum sensitivity and specificity will be reduced if reagents are modified or not stored under the recommended conditions.
- Do not mix different lots of reagents.
- Avoid microbial contamination of opened reagent bottles.
- Ensure that no cross contamination occurs between wells.
- It is essential for proper performance of the test that the enzyme-conjugated antibody is not allowed to contaminate other reagents and equipment.
- Ensure that kit components are not exposed to temperatures greater than 40°C.
- Solutions containing sodium azide should not be used for cleaning of equipment, especially washing devices (the peroxidase enzyme used in the kit is inactivated by sodium azide).
- Do not use for diagnostic purposes of medical specimens.

### 14. MSDS INFORMATION

Material safety data sheets (MSDS) are available for this test on request.

### 15. WARRANTY

Accurate results depend on the proper use of the kit by following the instructions for use carefully. If the kit fails to perform according to specification, please contact:

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## Summary of changes

Change date	Issue Number	Change Summary
Feb2020	1.0	New document
Nov2020	2.0	PerkinElmer rebranding and inclusion of instructions for both 1 and 5 plate kits.

NOTE: Minor changes (e.g. formatting, grammar, correcting typographical errors) are not included in the summary of changes.

For more information visit [www.solusscientific.com](http://www.solusscientific.com)

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