

# SOLUS *E. COLI* O157 ELISA

Immunoassay-Based Test System for  
the Detection of *E. coli* O157  
in Foods and Environmental Samples

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SOL 37/03-10/15  
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS  
<http://nf-validation.afnor.org/en>

This method is certified by AFNOR Certification for the detection of *E. coli* O157, including H7, in raw beef meat products (seasoned or not) raw milk and dairy products, vegetables and environmental samples (validation ref. no. **SOL 37/03-10/15**).

## 1. INTRODUCTION

Solus *E. coli* O157 ELISA provides a negative or a presumptive positive result from a single enrichment within 18 to 22 hours, including the assay time.

## 2. INTENDED USE

Solus *E. coli* O157 ELISA is for the detection of *E. coli* O157 in selected foods and production environmental 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 stabilised and ready to use at working concentration with only the Washing Buffer concentrate requiring dilution. Each kit contains sufficient material for 1 x 93 determinations, plus controls. The kit expiry date is displayed on each product label.

Component	Appearance	Volume	Comments
Assay plate	96-well microplate with removable/breakable strip format	1	Wells coated with antibodies against <i>E. coli</i> O157
Negative control	Colourless/clear liquid. Green label.	3ml	Working concentration. Contains diluent with preservative.
Positive control	Black liquid. Red label.	3ml	Working concentration. Contains heat-killed <i>E. coli</i> O157 in diluent with preservative.
Conjugate	Colourless/very pale straw-coloured liquid. Orange label.	11ml	Working concentration. Contains horseradish peroxidase antibody conjugate in diluent with preservative.
Substrate	Colourless/very pale blue liquid. Blue label.	11ml	Working concentration. Contains 3, 3', 5, 5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers.
Stop solution	Colourless liquid. Yellow label.	11ml	Working concentration. Contains 0.2M sulphuric acid.
Washing buffer concentrate	Colourless/yellow/orange liquid. White label.	10ml x 6	Concentrated. Dilute before use.
Frit filters	Flat, white plastic discs ~12mm diameter	100	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	Timer
Deionised or distilled (DI) water	Incubator at 37±1°C
Modified Tryptone Soya Broth + novobiocin (20mg/L)	Incubator at 41.5±1°C
Measuring cylinders for various volumes (e.g. 250 ml, 1L)	Heating apparatus (e.g. heat block) capable of heating to 85-100°C
Sterile 10ml test tubes suitable for selective enrichment	
Homogeniser (or similar apparatus) and filter bags	Pipettes and tips (1ml; 0.1ml)
3ml transfer pipettes (sterile)	Dynex DS2 or Microplate washer and microplate reader with 450nm filter
Vortex mixer	
Tubes for sample boiling (e.g. 5ml polypropylene rimless tubes 12x75mm)	Autoclave for decontamination of samples

## 5. REAGENT PREPARATION

### 5.1 Wash Buffer:

Add the contents of a Wash buffer bottle (10ml) to 240ml of deionised water to prepare the wash buffer at assay concentration. Dispense and label as appropriate. The prepared solution can be stored for a maximum of 4 weeks if kept at 2-8°C.

### 5.2 Culture Broth (growth medium):

Prepare the modified Tryptone Soya Broth following manufacturer's instructions. Allow to cool to ambient temperature (18-30°C) before adding the novobiocin supplement at 20mg/L (mTSBn).

## 6. SAMPLE PREPARATION AND ENRICHMENT- standard method

Homogenise 25g of the sample to be tested, if necessary by homogeniser, in 225ml Modified Tryptone Soya Broth (mTSB) + novobiocin (20mg/L) and incubate for 16-20 hours at 41.5±1°C. In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.

## 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 (18-30°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 (18-30°C).

The non-heat-inactivated samples should be kept for verification until ELISA results are obtained. These samples should be kept at 41.5±1°C if the ELISA 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 ELISA test.

## 8. ELISA ASSAY PROCEDURE

- 8.1. Take test kit from storage at least one hour before use to allow the components to reach ambient temperature (18-30°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.
- 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 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 (18-30°C) for 10 minutes in the dark.
- 8.14. After incubation, stop the reaction by adding 0.1ml of Stop Solution (Yellow label) to all wells including the 'blank' well. The stop solution will cause any blue colour 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<sub>450</sub>) measurements using a microplate reader.

Acceptance criteria:

Negative Control OD <sub>450</sub>	< 0.100
Positive Control OD <sub>450</sub>	> 0.500

The value of the blanking well (usually A1 when processing manually) should always be subtracted. 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<sub>450</sub> 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 wells with OD<sub>450</sub> ≥ 0.200 are considered presumptive positive for *E. coli* O157. Presumptive positive results must be verified using a recognised culture method.

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

In the context of NF VALIDATION, all samples identified as positive by the alternative method must be confirmed by one of the following tests:

- Using the conventional tests described in the standardised methods by CEN or ISO. The confirmation step must start from the non-heat-inactivated mTSB+n broth stored at 41.5°C or 2-8°C. (See ISO 16654:2001 Horizontal method for the detection of *Escherichia coli* O157).
- Streaking mTSB+n (10µL) onto CT-SMAC and a chromogenic agar plate (such as CHROMagar O157) and incubate the plates at 37°C±1° for 18-24 hours. Perform a serological identification of characteristic colonies, with or without a purification step, using an O157 latex test (Microgen *E. coli* O157 M44) and after a purification step using H7 latex test (Wellcolex *E. coli* O157:H7 R30959601).

In the event of discordant results (presumptive positive ELISA results and a negative culture result) an IMS step must be used as a confirmation step by taking 1 ml of mTSB+n (non-heated) and follow the method as described in ISO 16654:2001 prior to streaking the magnetic particles onto both agar plates. This method is more sensitive than direct plating and can help to confirm samples that contain lower levels of target organism.

In the event of discordant results (presumptive positive ELISA result non-confirmed by one of the means described above and in particular for the latex tests) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

## 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 4 weeks 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 sulphuric 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

- 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
Jan2020	1	Rebrand of Solus E.coli O157 ELISA – QCF49 – Issue 1.3 – 07/16.
Apr2020	2	Addition of black dye to positive control and inclusion of frit filters in assay kit.

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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