

SOL 37/02-06/13

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

<http://nf-validation.afnor.org/en>

Solus *Listeria* ELISA

ELISA Based Test System For the Detection of *Listeria* in Foods and Environmental Samples

Product Number: LIS-0480S

This method is certified by AFNOR Certification for the detection of *Listeria* in food and feed products (validation ref no. SOL 37/02 – 06/13 valid until 18/06/2021).

1. INTRODUCTION

Solus *Listeria* ELISA provides a negative or a presumptive positive result including 2 enrichment steps within 44 to 52 hours. The ELISA part can be performed in less than 2 hours.

2. INTENDED USE

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).

The detection limit of the kit is in the range of 10^5 – 10^6 cells per ml in the enrichment broth.

3. REAGENTS PROVIDED

Kit components are supplied stabilised and ready to use at working concentration. Only the Washing Buffer has to be diluted 25 times in deionised water.

Each kit contains sufficient material for 5 x 93 determinations, plus controls.

The kit expiry date is displayed on each product label.

• 5 x 96 well microplate (in breakable strip format). Wells are coated with antibodies against *Listeria* sp.

• Negative Control (Green label). 10ml in working dilution. Contains diluent with preservative.

• Positive Control (Red label). 10ml in working dilution. Contains heat-killed *Listeria* in diluent with preservative. ***Positive Control is black in colour***

• Conjugate (Orange label). 60ml in working dilution. Contains horseradish peroxidase-antibody conjugate in diluent with stabilisers.

• Substrate (Blue label). 60ml in working dilution. Contains 3,3',5,5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers. Solution should be clear or slightly faint blue solution.

• Stop Solution. (Yellow label). 60ml in working dilution. Contains 10% sulphuric acid.

• Washing Buffer Concentrate (25x). 5 x 60ml. Contains 0.25M Tris-HCl/3.5M NaCl/ 1.25% Tween 20 and preservative, pH 8.0.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Refrigerator at 2-8°C.
 - Deionised or distilled water
 - Half-Fraser broth
 - Fraser broth
 - RELM selective broth
 - Measuring cylinder for 250ml or 1L
 - Sterile 10ml test tubes suitable for selective enrichment culture
 - Stomacher (or similar apparatus) and bags
 - 3ml transfer pipettes (sterile)
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- Test Tube for sample boiling (e.g. 5ml Polypropylene rimless test tubes 13 x 75mm)
- Vortex mixer
- Timer
- Incubator at 37±1°C
- Incubator at 30±1°C
- Water bath (capable of heating to 85-100°C)
- Pipettes and tips (1ml; 0.1ml)
- Microplate washer or squeeze bottle
- Microplate reader with 450 nm filter
- Autoclave for decontamination of samples

5. REAGENT PREPARATION

5.1. Wash Buffer:

Add the contents of a Wash buffer bottle (60ml) to 1500ml of deionised or distilled water to prepare the wash buffer at assay concentration.

Dispense and label as appropriate.

5.2 Culture Broth (growth medium):

Prepare Half-Fraser following manufacturer's instructions. Allow to cool to room temperature before use in testing.

Prepare RELM broth and dispense in 10ml tubes following manufacturer's instruction. Allow to cool to room temperature before use in testing.

6. SAMPLE PREPARATION AND ENRICHMENT- standard method

Pre-enrichment

X grams of the sample to be tested is homogenised, if necessary by stomacher, and incubate for 24±2hours in 9*X ml of Half Fraser at 30±1°C.

In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.

Selective enrichment

0.2ml of the enriched sample is transferred to 10 ml of RELM broth and incubated for 24±2hours at 30°C±1°C. Ensure that the bench processing time of inoculated samples is kept to a minimum (when processing a large number of samples, transfer the samples to tubes in individual racks, when each rack

is completed transfer immediately to the incubator. This is important to avoid extensive growth of competing non-*Listeria*).

When the incubation period in RELM is completed, agitate the tubes gently and transfer 1ml aliquot to a glass or polypropylene test tube.

The un-boiled RELM samples should be kept for verification until ELISA results are obtained. These samples should be kept at 30±1°C if the ELISA test is to be carried out within 2 hours or at 2-8°C if not. It is possible to keep RELM broths for 72 hours at 2-8°C prior to the ELISA test.

Heat the aliquot to 85-100°C for 15-20 minutes in the test tube. After heating, allow the sample to cool to room temperature. This may be accelerated by placing the test tubes in cold tap water for 5 minutes.

7. ELISA ASSAY PROCEDURE

7.1. Take the test kit from storage at 2-8°C one hour before use to allow the components to reach room temperature. Determine the number of wells required for the test. Take the 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.

7.2. Prepare Wash Buffer as detailed in section 5.1.

7.3. Leave the first well in the strip empty to serve as a 'blank' for measuring the absorbance of the substrate.

7.4. Pipette 0.1ml of Negative Control (Green label) into the second well.

7.5. Pipette 0.1ml of Positive Control (Red label, black in colour) into the third well.

7.6. Pipette 0.1ml of each boiled cooled 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.

7.7. Incubate the plate (containing the strips) at 37±1°C for 30mins. (±5 mins.).

7.8. 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.

7.9. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.

7.10. Incubate the plate at $37 \pm 1^\circ\text{C}$ for 30mins. (± 5 mins.).

7.11. Repeat the wash cycles as detailed in section 7.8

7.12. Pipette 0.1ml of TMB Substrate (Blue label) into all wells, including the 'blank' well.

7.13. Incubate the plate at room temperature for 30 mins. (± 5 mins.).

7.14. 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 the blue colour in wells to change to yellow.

7.15. The optical densities need to be read within 10 minutes in a plate reader using a 450nm filter. Inspect the wells before reading 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.

8. INTERPRETATION OF RESULTS

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

Assay acceptance criteria:

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

Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be performed again. Typical values for the negative control are 0.050 and for the positive control 0.900-1.700. The value of the blanking well (usually A1) should always be subtracted.

Samples with OD_{450} readings of less than

0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding non-boiled aliquot of RELM broth may be discarded following local regulations/guidelines.

Sample wells with $\text{OD}_{450} \geq 0.200$ are considered presumptive positive for *Listeria*. Presumptive positive results must be verified using a recognised culture method.

9. CONFIRMATION OF POSITIVE RESULTS FROM *LISTERIA* ELISA

Samples with OD 's ≥ 0.200 are considered positive for *Listeria*.

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

- Isolate (from unheated RELM stored at 30°C or $2-8^\circ\text{C}$) on one *Listeria* selective agar plate (ISO formulation of OAA, Palcam or Oxford), then use the *Listeria* genus conventional tests described in the standardised methods by CEN or ISO including the purification step.

- Isolate (from unheated RELM stored at 30°C or $2-8^\circ\text{C}$) on one *Listeria* selective agar plate (ISO formulation of OAA or Palcam) then perform confirmation tests (Gram and catalase).

It is possible to perform confirmation tests directly (without prior purification) if the colonies are well isolated (one to five colonies are to be tested for confirmation).

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

For example performing further streaking of the retained broth culture and possibly a re-test of the original food sample to ensure the quality of the result.

10. 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 at 2-8°C for up to 10 days.

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.

11. SAFETY

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.

While the procedures detailed are simple and easy to perform, they require laboratory facilities with qualified and trained personnel for the handling of potentially pathogenic organisms. Training is recommended to first time users and is provided by Solus Scientific Solutions Ltd. As a guide, the following precautions should be taken as a minimum:

- Protective clothing should be worn including safety glasses, mask and gloves where appropriate.
- Do not pipette by mouth.
- Avoid contact with the skin.

12. 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.
- It is not recommended to use solutions containing sodium azide 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.

13. SDS INFORMATION

Safety data sheets (SDS) are available for this test on request.

14. 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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