

SOLUS *LISTERIA* ELISA

Immunoassay-Based Test System for
the Detection of *Listeria* spp.
in Foods and Environmental Samples

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SOL 37/02-06/13
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
<http://nf-validation.afnor.org/en>

This method is certified by AFNOR Certification for the detection of *Listeria* spp. in all human food products (by performing validation assays on a broad range of foods), and production environment samples (validation ref. no. **SOL 37/02-06/13**).

1. INTRODUCTION

Solus *Listeria* ELISA provides a negative or a presumptive positive result from 2 enrichment steps within 44 to 52 hours, including the assay time.

2. INTENDED USE

Solus *Listeria* ELISA is for the detection of *Listeria* spp. 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 (LIS-0096S) or 5 (LIS-0480S) x 93 determinations, plus controls. The kit expiry date is displayed on each product label.

Component	Appearance	Volume		Comments
		LIS-0096S	LIS-0480S	
Assay plate	96-well microplate with removable/breakable strip format	1	5	Wells coated with antibodies against <i>Listeria</i> spp.
Negative control	Pale orange 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>Listeria</i> in diluent with preservative.
Conjugate	Colourless/very pale straw-coloured liquid. Orange label.	11ml	60ml	Working concentration. Contains horseradish peroxidase antibody conjugate in diluent with preservative.
Substrate	Colourless/very pale blue liquid. Blue label.	11ml	60ml	Working concentration. Contains 3, 3', 5, 5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers.
Stop solution	Colourless liquid. Yellow label.	11ml	60ml	Working concentration. Contains 0.2M sulphuric acid.
Washing buffer concentrate	Colourless/yellow/orange liquid. White label.	10ml x 6	60ml x 5	Concentrated. Dilute before use.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Refrigerator at 2-8°C	Timer
Deionised or distilled (DI) water	Incubator at 37±1°C
Half Fraser broth	Incubator at 30±1°C
RELM selective broth	Tubes for sample boiling (e.g. 5ml polypropylene rimless tubes 12x75mm)
Sponge samplers or swabs soaked in suitable neutralizing buffer (e.g. Lethen broth or HiCap buffer)	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)
Sterile 10ml test tubes suitable for selective enrichment	Dynex DS2 or Microplate washer and microplate reader with 450nm filter
Homogeniser (or similar apparatus) and bags	Autoclave for decontamination of samples
3ml transfer pipettes (sterile)	Agar plates and standard confirmation test equipment.
Vortex mixer	See section 10 for details.

5. REAGENT PREPARATION

5.1 Wash Buffer:

Prepare the following in a clean vessel.

LIS-0096S	LIS-0480S
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 Half Fraser broth following manufacturer's instructions. Allow to cool to ambient temperature (18-25°C) before use in testing.
- Prepare RELM broth following manufacturer's instruction and dispense into sterile 10ml tubes. Allow to cool to ambient temperature (18-25°C) before use in testing.

6. SAMPLE PREPARATION AND ENRICHMENT- standard method

6.1 Pre-enrichment (all samples but environmental surfaces)

- Homogenise Xg of the sample to be tested, if necessary by homogeniser, in 9*Xml of Half Fraser broth and incubate for 22-26 hours at 30±1°C. In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.

6.2 Pre-enrichment for environmental surfaces

- Use sterile swabs, sponges or wipes premoistened in neutralising broth. Sample the environmental surface then enrich swab in 10ml, sponge in 100ml or wipes in 225ml of Half Fraser broth for 22-26 hours at 30±1°C.

6.3 Selective enrichment

- Transfer 0.2ml of the enriched sample into 10ml of RELM broth and incubate for 22-26 hours at 30±1°C.

Ensure that the bench processing time of samples is kept to a minimum and that transfer to 30°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.

The non-heat-inactivated 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. 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 (18-25°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 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 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_{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. 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 wells with $OD_{450} \geq 0.200$ are considered presumptive positive for *Listeria*. Presumptive positive results must be verified using a recognised culture method.

10. CONFIRMATION OF POSITIVE RESULTS FROM *LISTERIA* 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 including the purification step. The confirmation step must start from the non-heat-inactivated RELM broth stored at 30°C or 2-8°C.
- Streaking RELM (10µL) onto 1 agar plate (ISO formulation of OAA, Palcam or Oxford). Incubate agars as specified by standard *Listeria* cultural protocols then perform confirmation tests: Gram stain 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. The presence of characteristic colonies on selective media is sufficient to confirm the presence of *Listeria* spp.

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.

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 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 and combining of Solus Listeria ELISA – QCF 18 – Issue 1.4 – 08/17 and Solus Listeria ELISA – QCF 19 – Issue 1.6 – 08/17
Feb2021	2	Amendments to 10. CONFIRMATION OF POSITIVE RESULTS FROM LISTERIA ELISA. Amendments to 6.2 SAMPLE PREPARATION AND ENRICHMENT- standard method

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

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