BAC*Spec Listeria*

ELISA TEST KIT FOR QUALITATIVE DETECTION OF *LISTERIA*

Cat. No. 4323410201 and 4323410205

For 1 x 96 well plate
or 5 x 96 well plate

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1 INTRODUCTION

The rapid detection of pathogens in foods is essential for ensuring the safety of consumers. Traditional methods for detection of foodborne bacteria use time consuming growth in culture media followed by isolation, biochemical identification and serology.

ELISA detection and identification is faster, more convenient and more specific than conventional methods. This ELISA assay gives a presumptive result after a simple and efficient 44 hour two step enrichment procedure. The ELISA can be performed in less than two hours; the presumptive results can be obtained within 46 hours.

1.1 Certifications

1.1.1 AFNOR Certification

The BAC*Spec Listeria* kit is certified by AFNOR Certification as an alternative method for *Listeria* spp. detection in all human food products (25 g / 25 mL) and environmental samples according to EN ISO 16140-2.

The end of the validity of the NF VALIDATION certification is indicated on the certificate EGS 38/04-01/17.

1.1.2 AOAC Certification

The BAC*Spec*™ *Listeria* kit was reviewed by AOAC Research Institute and was found to perform to the manufacturer´s specifications.

The kit is thus certified by AOAC-Research Institute under the Performance Tested Methods[SM] Program for detection of *Listeria* in:

- 25 g / 25 mL samples of
  - Frankfurters
  - Mayonnaise-based vegetable salad
  - Soft white cheese
  - Frozen shrimp
  - Smoked salmon
  - Raw milk
  - Frozen cantaloupe balls
  - Process water
- Stainless steel environmental surface
- Ceramic environmental surface

The end of the validity of the AOAC validation is indicated on the certificate 051703.

2 INTENDED USE

The BAC*Spec*™ *Listeria* detection kit provides materials for rapid in vitro detection of *Listeria* spp. (included in NF VALIDATION scope and AOAC validated for: *L. monocytogenes*, *L. grayi*, *L. innocua*, *L. ivanovii*, *L. welshimeri* and *L. seeligeri*; additionally AOAC validated for *L. marthii* and *L. rocourtiae*) from human food products and environmental samples. *Listeria* serotypes which are motile will give positive signals with this test kit.

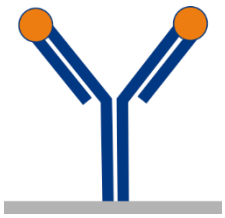
The BAC*Spec*™ *Listeria* detection kit is intended to be used in analytical laboratories for testing of human food products and environmental samples from food production facilities. It may also be applied for other purposes in food product research and e.g. microbial monitoring of production processes. The kit is not intended for diagnostic use with medical specimens.

3 PRINCIPLE OF THE ASSAY

Food and environmental samples are enriched in selective pre-enrichment buffer followed by secondary enrichment in a selective medium. An aliquot of the selective enriched sample is withdrawn and heat-treated. After cooling, the samples are investigated for the presence of *Listeria* antigens with a sandwich ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay) method.

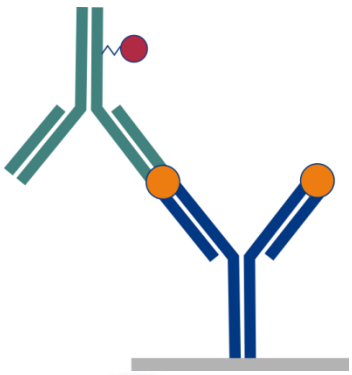
1. Affinity purified antibody specific for *Listeria* flagella protein is bound to the wells of microtiter strips.

2. The heat treated samples, containing the bacterial protein antigens, are cooled to room temperature (20-25°C) and added to the antibody coated wells. *Listeria* protein antigens present in the samples are bound immunologically by the antibody.

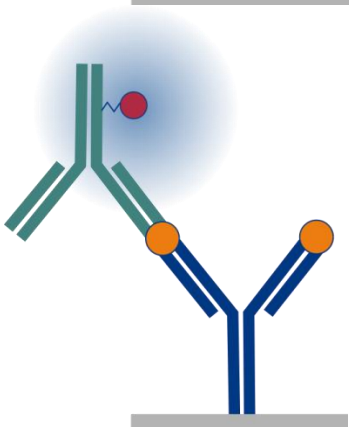


3. After washing to remove unbound material, enzyme-conjugated affinity purified antibodies which are specific for *Listeria* proteins are added to the wells.

   If *Listeria* proteins are present in the samples, the enzyme-conjugated antibodies bind to the proteins and thus to the well.



4. After a second washing step where any unbound enzyme-conjugated antibody is removed, enzyme substrate is added. A blue colour is formed by the action of bound enzyme on the substrate in those wells where *Listeria* proteins are present. The reaction with the substrate is stopped after 30 minutes with diluted sulphuric acid which changes the colour in the wells to yellow.



4 REAGENTS PROVIDED

The ELISA test kit contains 96 well microtiter plate(s), which can be separated into single wells. Each kit contains sufficient reagents for the microtiter wells/plates included in the package. The kit expiration date is indicated on the label. Store the kit and unused kit components at 2 - 8 °C protected from light. Do not freeze.

ELISA Plate(s)

The microtiter plate(s) consist of a frame with 12 strips of 8 wells coated with antibodies against *Listeria*. The microtiter plate is sealed into an aluminium foil bag with a desiccant pack. Return unused wells to the aluminium foil bag with the desiccant pack and close the bag.

- cat no -01: 1 microtiter plate, 96 wells
- cat no -05: 5 microtiter plates, 480 wells

Negative Control (green colour code)

Contains diluent with stabiliser in working dilution.

- cat no -01: 1 vial with 3 mL
- cat no -05: 1 vial with 10 mL

Positive Control (red colour code)

Contains inactivated *Listeria* in diluent with stabiliser in working dilution.

- cat no -01: 1 vial with 3 mL
- cat no -05: 1 vial with 10 mL

Conjugate (orange colour code)

Contains horseradish peroxidase-antibody conjugate in diluent with stabilisers in working dilution.

- cat no -01: 1 vial with 12 mL
- cat no -05: 1 vial with 60 mL

Substrate (blue colour code)

Contains 3,3',5,5'-tetramethylbenzidine (TMB) in working dilution. Clear or faint blue solution. Avoid exposure to sunlight or any other strong light.

- cat no -01: 1 vial with 12 mL
- cat no -05: 1 vial with 60 mL

Stop Solution (yellow colour code)

Contains 0.2 M sulphuric acid.

- cat no -01: 1 vial with 12 mL
- cat no -05: 2 vials with 30 mL

Washing Buffer Concentrate

Contains 0.075 M Tris-HCl/2.5 M NaCl with 5% Tween 20, pH 7.2

- cat no -01: 6 vials with 10 mL each.

  Dilute each vial 25-fold with 240 mL of deionised or distilled water. 250 mL of diluted washing buffer is sufficient for 2 strips through both washing procedures of the test.

- cat no -05: 5 vials with 60 mL each.

  Dilute each vial 25-fold with 1440 mL of deionised or distilled water.

Diluted Washing Buffer may be stored for up to 1 week at RT protected from light.

Cover-foil for plate incubation

5 REAGENTS AND EQUIPMENT NOT SUPPLIED WITH THE KIT

1. Deionised or distilled water
2. Micropipette for dispensing 100 µL
3. Tips for micropipette
4. Microtiter Plate Washer
5. Air incubator for 30 °C, accuracy ±1 °C, and air incubator for 37 °C, accuracy ±1 °C
6. Micro-well Plate Reader with 450 nm filter
7. Stopwatch
8. Measuring cylinder for 250 ml
9. Sterile flasks, stomacher bags or jars suitable for enrichment culture
10. Swabs / sponges / wipes for environmental samples
11. Sterile 10 mL test tubes suitable for selective enrichment culture
12. Sterile transfer pipettes approximately 1 mL for transfer of aliquots to tubes for heat-treatment
13. Block Heaters or water bath for 85-100°C, accuracy ±5 °C
14. Heat resistant tubes with caps
15. Autoclave for disinfection of samples
16. Stomacher for homogenisation of samples
17. Half Fraser Broth for pre-enrichment
18. Eurofins *Listeria* Enrichment Broth (ELEB) for secondary enrichment (LabM, cat. no. LAB589-AGS)
19. MALDI biotyper of Bruker (software version: Flex Control 3.4, Flex Analysis 3.4 MBT Compass explorer 4.1)
20. (optional) Automated ELISA analyser, which can fulfil the test conditions.

6 WORKING GUIDELINES AND PRECAUTIONS

6.1 Safety Precautions

- Warning: The Stop Solution contains sulphuric acid which is corrosive to metal, (Category 1), H290.
- *Listeria* monocytogenes should not be handled by pregnant women, children, the elderly and immunocompromised individuals due to the high infection risk and fatal health consequences for this group, in particular for the unborn child in case of pregnant women.
- The *Listeria* in the positive control have been proven to be non-viable. The positive control should nonetheless be treated as potentially hazardous.
- All samples should be handled with caution as they are potentially infectious.
- Do not eat, drink or apply cosmetics in the work area where the test is performed.
- Do not pipette by mouth.
- Avoid contact of kit components with injured skin.

6.2 Working Guidelines

- Comply with Good Manufacturing Practice (refer to EN ISO 7218 standard)
- All work should be performed by trained personnel in laboratories meeting Biosafety Level 2 (BSL2) regulations.
- Components must not be used after the expiration date printed on the label.
- Reagents except the Washing Buffer 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.
- The kit should be stored at 2-8°C. Ensure that kit components are not exposed to temperatures higher than 40°C.
- Shelf life is indicated on the labels of the components.

6.3 Waste Disposal

- Dispose any waste which is potentially contaminated with pathogenic bacteria according to your internal and local regulations.

7 PREPARATION PRIOR TO ELISA

7.1 Preparation of the Enrichment Media

Prepare liquid Half Fraser medium according to instructions provided by the manufacturers. After autoclaving, allow the medium to cool to room temperature (20-25°C) before applying it to the samples.

Eurofins *Listeria* Enrichment Broth (ELEB) for the secondary enrichment is also prepared according to the instructions of the manufacturer. Dispense 10 mL into the size-appropriate sterile tubes. Allow this to cool before applying to the sample.

7.2 Preparation of Samples, Standard Enrichment Protocol

Pre-enrichment:

- Homogenise 25 g / 25 mL of food sample, if necessary by stomacher, in 225 mL of Half Fraser medium.
- For enrichment of environmental samples follow instructions of EN ISO 18593 standard.
- Environmental samples should be prepared as follows:

Sample Preparation of Environmental Samples	
Sample Type	**Volume of Half Fraser Medium**
25 g / 25 mL (e.g. dusts, process water)	225 mL
1 swab	10 mL
1 wipe	225 mL
1 sponge	100 mL

- Incubate the samples for 22 - 26 hours at 30 ± 1°C.

Selective Enrichment:

Transfer 0.2 ± 0.01 mL of the enriched sample to 10 mL of ELEB and incubate for 22 - 26 hours at 30 °C ± 1 °C in an incubator or water-bath. Use pipettes and incubators of required accuracy. Ensure that the bench time of inoculated samples is kept at a minimum. When processing large numbers of samples, transfer inocula to tubes in individual racks and transfer single racks immediately to incubator when ready. This is important to avoid extensive growth of competing micro-organisms.

When the incubation period in ELEB is completed, agitate the tubes gently and transfer a 0.5 - 1 mL aliquot to a glass or polypropylene test tube. Alternatively, store the ELEB at 2 - 8 °C for up to 72 hours before sampling. After sampling, the un-heated enrichment samples should be kept at 2 - 8 °C for verification until ELISA results are obtained. Heat the aliquot to 85 - 100 °C for 15 - 20 minutes in the closed test tube. Cool the samples to room temperature (20-25°C). This may be done by placing the test tube in cold tap water for 5 minutes.

8 PROCEDURE OF THE ELISA TEST

Remove the test kit from cold storage (2 - 8 °C) one hour before use to allow the components to warm to room temperature (20 – 25°C).

STEP 1

1. Determine the number of wells required for the test. Take the required number of strips/wells from the bag and fit them to the frame provided. Unused strips/wells should be returned to the bag and stored at 2 - 8 °C.
2. The first well in the strip is reserved as a 'blank' for measuring the absorbance of the substrate and stop solution.
3. Pipette 0.1 mL of negative control into the second well (B1).
4. Pipette 0.1 mL of positive control into the third well (C1).
5. Pipette 0.1 mL of each heated and 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.
6. Close the frame containing the strips with the cover foil and incubate for 30 minutes at 37 °C ± 1 °C. A prolongation up to 5 minutes is tolerated. Proceed immediately with the next step.
7. If not yet done, dilute the Washing Buffer Concentrate 25-fold with distilled or deionised water as described above.
8. Wash the wells 5 - 7 times with diluted washing buffer. Preferably use a washing device. The washing technique is critical for the assay performance. Ensure complete filling and clearing of the wells through all steps of each washing cycle. At the end of the last cycle, remove as much liquid as possible.

STEP 2

9. Immediately after the washing procedure, pipette 0.1 mL of conjugate into all wells except the 'blank' (A1).
10. Close the frame with the Cover-foil and incubate for 30 minutes at 37 °C ± 1 °C. A prolongation up to 5 minutes is tolerated.
11. Wash the wells 5 - 7 times with diluted washing buffer as described above.

STEP 3

12. Pipette 0.1mL of TMB substrate into all wells, including the 'blank' well (A1).
13. Incubate the wells with substrate for 30 minutes at room temperature (20-25°C). (Start stopwatch at well A1)

STEP 4

14. Stop the colour reaction after 30 minutes by adding 100 µL of stop solution to all wells including the 'blank' well. The stop solution will cause the blue colour to change to yellow in wells with positive samples.
15. Read optical densities (OD) within 10 minutes in a micro-well reader with a 450 nm filter. Do not use reference filter. The photometer should be zeroed against the 'blank' well (usually A1) before the other wells are read. Alternatively, subtract the value of the blank well from the measured value for each sample manually after measuring.

When the user is familiar with the procedure, the Short Instructions description on the back of this booklet may be followed. Automatic ELISA equipment is allowed if the prescribed protocol is followed.

9 INTERPRETATION OF RESULTS

Results are measured with the help of a micro-well reader and are expressed precisely as optical density (OD). Subtract the value of the blank well (usually A1) from the measured value for the final result of both, controls and samples.

9.1 Controls

The OD reading of the Negative Control must be below 0.100 and the OD reading of the Positive Control must be above 0.500.

Remember to subtract the value of the blank well (usually A1).

9.2 Result Interpretation for Samples

Samples from wells with OD values above 0.275 are considered positive for *Listeria*. Remember to subtract the value of the blank well (usually A1).

10 CONFIRMATION OF PRESUMPTIVE POSITIVE RESULTS

In the context of NF VALIDATION by AFNOR Certification, all samples identified as positive by the BAC*Spec Listeria* ELISA kit must be confirmed by (one of) the following test(s):

Option 1:

Using the conventional tests described in the methods standardized by CEN or ISO from colonies (including the purification step). The confirmation step must start from the enrichment broth or from typical colonies isolated on selective media.

Option 2:

Direct streaking of 10 µl from enrichment in Eurofins *Listeria* Enrichment broth (ELEB) onto O&A and PALCAM agars. Incubation of the O&A plates at 37°C ±1°C for 24 ±3h and if necessary (negative) further 24h ±3h. Incubation of PALCAM plates at 37°C ±1°C for 48 ±3h. Check for the presence of characteristic colonies which are presumed to be *Listeria* spp. The presence of characteristic colonies is sufficient to confirm the presence of *Listeria* spp.

Option 3 (for NF VALIDATION, only):

Using the MALDI biotyper of Bruker (software version: Flex Control 3.4, Flex Analysis 3.4 MBT Compass explorer 4.1) for confirmation on isolated characteristic colonies which are presumed to be *Listeria* on O&A or PALCAM agars. If characteristic colonies are not well isolated, confirmation may also be performed after purification on TSYEA plates. This option is dedicated to the confirmation of *Listeria* spp. The identification of colonies is not part of the NF VALIDATION scope.

Option 4 (for NF VALIDATION, only):

Using any other method certified according to NF VALIDATION, the principle of which must be different from the BAC*Spec Listeria* ELISA kit. The protocol of detection of the second validated method used for the confirmation shall be followed entirely. All steps which are before the step from which the confirmation is started shall be common to both methods. The BAC*Spec Listeria* ELISA kit and the second validated method must have common first steps, for instance the same enrichment broth.

Independent of the confirmation method used, in the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

11 PERFORMANCE LIMITATIONS AND RECOMMENDATIONS FOR USE

Proper performance of the enrichment phase is important for the assay. Use Eurofins GeneScan Technologies recommended media for optimal performance. Other media may cause reduced performance of the kit. Test performance has been optimised using the culture reagents specified above and it is strongly recommended that only these media be used.

Ensure correct temperature setting of the incubator used for selective enrichment using a thermometer calibrated to a certified standard. Washing of the wells is a critical step. Do not attempt to use squeeze bottles or other uncontrolled devices. Use multichannel pipettes or preferably multichannel washing devices designed for micro-plates. Automatic devices give optimal results.

The enzyme peroxidase used in the kit will be inactivated in the presence of sodium azide. Therefore ensure that residues of sodium azide are not present in washing devices, buffer reservoir, tubes or the immuno-washer.

Performance data are provided on request.

12 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

Eurofins GeneScan Technologies GmbH (“GeneScan”) warrants the products manufactured by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions before the expiration date marked on the product packaging and when stored under the storage conditions recommended in the instructions and/or on the package. GeneScan makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

GeneScan’s sole obligation with the respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies GeneScan promptly of any such defect. GeneScan shall not be liable for any direct, indirect or consequential damages resulting from economic loss or property damages sustained by buyer or any customer from the use of the product(s). A copy of Eurofins GeneScan Technologies GmbH terms and conditions can be obtained on request, and is also provided in our price lists.

13 PRODUCT USE LIMITATIONS

This kit is developed, designed, and sold for research purposes only. It is not to be used for diagnostic purposes or analysis of food and feed unless expressly cleared for that purpose by the competent regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

14 IMPORTANT NOTES

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

15 ENRICHMENT SCHEME

	Enrichment Procedure	Recommendations
STEP A	Homogenize 25 g sample by stomaching in 225 mL Half Fraser Broth	Half Fraser should be at room temperature (20-25°C)
	Incubate for 22 - 26 h at 30 °C ± 1 °C.	
STEP B	Transfer 0.2 mL to 10 mL Eurofins *Listeria* Enrichment Broth (ELEB)	ELEB should be at room temperature (20-25°C). Do not over-inoculate. Limit handling time of ELEB inoculated samples. Higher numbers of samples should be split and each portion should be transferred to the incubator/water-bath without delay.
	Incubate for 22 - 26 h at 30 °C ± 1 °C	
STEP C	Re-suspend samples and transfer 0.5 – 1 mL to glass or polypropylene vials. Use closures or heat resistant foil to cover the vials. Heat at 85 – 100 °C for 15 – 20 minutes. Cool to room temperature (20 – 25°C) prior to ELISA.	Always keep the heat treated ELEB samples until the ELISA result is approved. Heat treated samples may be stored at 2 – 8 °C for 1 week. Un-heated samples can be stored at 2 – 8 °C up to 72 h for subsequent confirmation test(s).

16 ELISA PROCEDURE SCHEME

STEP 1	Well A1 is reserved as blank control 0.1 mL **negative control** into well B1 0.1 mL **positive control** into well C1 0.1 mL of each heated and cooled sample
Incubation:	*30 minutes at 37 °C ± 1 °C* *(a prolongation of up to 5 minutes is tolerated)*
Washing:	Wash the wells with diluted washing buffer

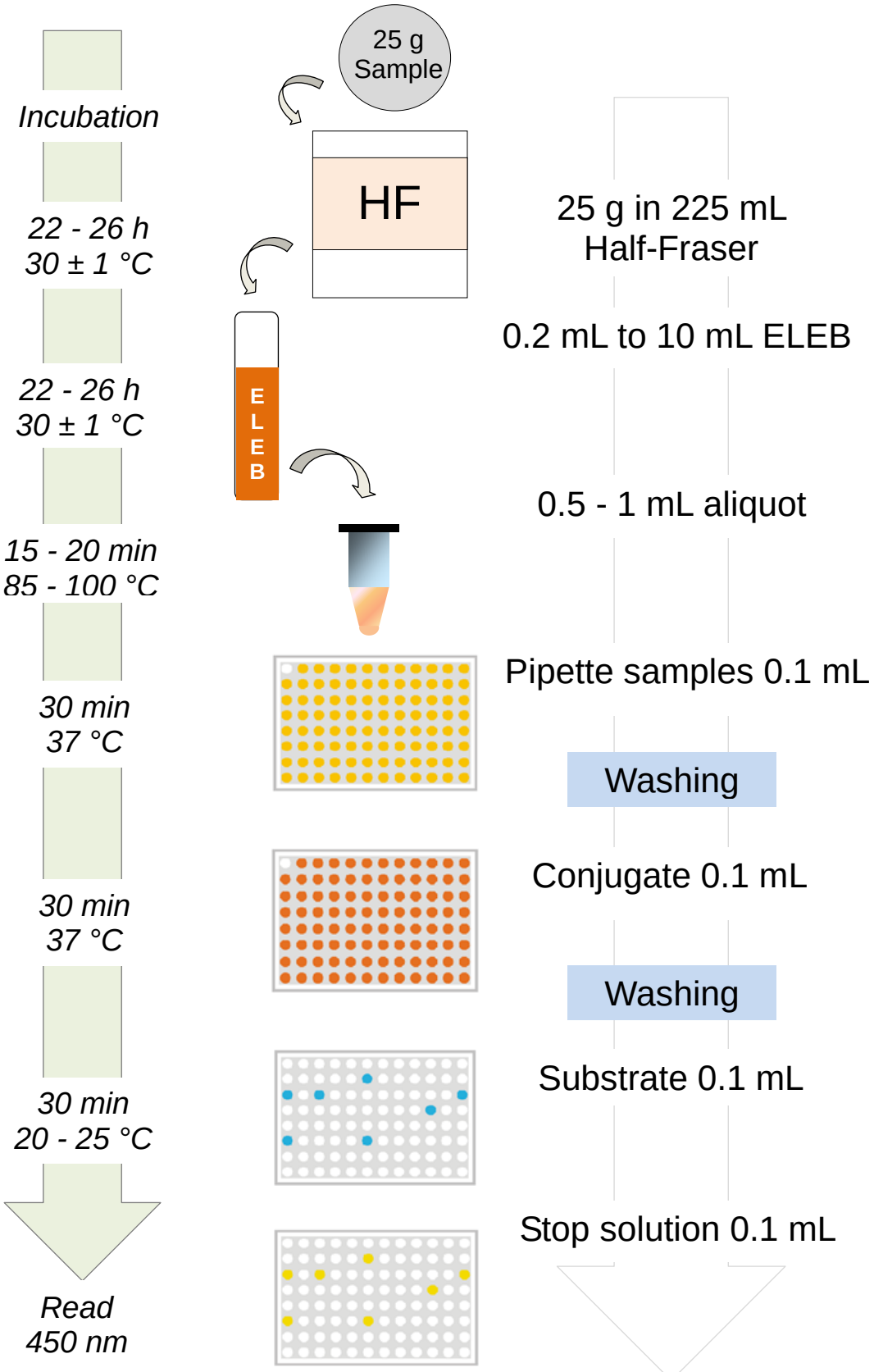
STEP 2	0.1 mL **conjugate** into all wells except blank (A1)
Incubation:	*30 minutes at 37 °C ± 1 °C* *(a prolongation of up to 5 minutes is tolerated)*
Washing:	Wash the wells with diluted washing buffer

STEP 3	0.1 mL **substrate** into all wells including blank (A1)
Incubation:	*30 minutes at room temperature (20-25°C)* *(a prolongation of up to 1 minute is tolerated)*

STEP 4	0.1 mL **stop solution** into all wells including blank (A1)
Read:	Read immediately or within 10 minutes at 450 nm

BAC*Spec*™ *Listeria*

SHORT INSTRUCTIONS



TECHNICAL SUPPORT SERVICE

For technical assistance and more information please contact the Eurofins GeneScan Technologies GmbH Customer Service or your local distributor.

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