hygiena®

BAX® System X5

PCR Assay for *L. monocytogenes*

Part KIT2023

KIT CONTENTS

64 PCR tubes with tablets (8 x 8 strips)
96 flat optical caps (12 x 8 strips)
1 bottle of protease (400 µL)
2 bottles of lysis buffer (12 mL)
1 bottle Lysing agent 2 (1.1 mL)

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

QUA 18/05 -07/08
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE
LICENSE NUMBER 070202

INTENDED USE

Food processors and associated laboratories can use the BAX® System X5 as a quick and reliable method for detecting *Listeria monocytogenes* from a variety of foods. This assay is designed to report yes/no results for *Listeria monocytogenes* at concentrations as low as 10^4 cfu/mL after enrichment. With a processing time of approximately 3.5 hours in the BAX® System X5 instrument, the method returns results comparable to culture methods, but with a significantly faster time to result.

BAX® Systems are designed for use by qualified lab personnel who follow standard microbiology laboratory practice, including the safe handling and disposal of potentially pathogenic materials. The laboratory must comply with good laboratory practice (see ISO 7218 standard).

Field of use: Data obtained from the BAX® System should not be used for human diagnostic or human treatment purposes. Equipment is not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. The BAX® System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under supervision of technically qualified persons.

PRINCIPLE OF THE METHOD

See the BAX® System X5 User Guide for an overview of how the BAX® System method uses automated, Polymerase Chain Reaction (PCR) technology.

MATERIALS

BAX® System X5 PCR Assay for *L. monocytogenes* (Part KIT2023

BAX® System X5 start-up package (equipment and supplies)

- BAX® System X5 instrument and associated computer
- Automated Thermal Block*
- 32-well aluminum cold block with insulator
- 32-well PCR tube holder
- Capping/decapping tools
- Adjustable pipettes (5-50 µL; 20-200 µL)
- Repeating pipette
- Multi-channel pipette (8 channels – 5-50 µL)
- Cluster tubes with caps and racks
- Pipette tips with barriers
- Powder free nitrile gloves

**Analog heating and cooling blocks may be used in place of the Automated Thermal Block. See the BAX® System X5 User Guide for details.*

Stomacher with bags

Incubators capable of maintaining directed temperatures within ±2°C.

***Note**: Health Canada, AOAC and AFNOR Certification standards require an incubator capable of maintaining ±1°C.*

Enrichment media (See BAX® System X5 User Guide for details)

- 24 LEB Complete – Catalog No. MED2005 or Oxoid CM1154.
- 24 LEB Buffer Supplement– (required by AFNOR only for smoked fish and charcuterie) -Catalog No. MED2000 or Oxoid BO1204M/BO1204E.

  ***Note**: 24 LEB buffer supplement may also be beneficial for other samples that experience a drop in pH during enrichment. Before testing any food types that have not been certified by AFNOR or AOAC, it is strongly recommended that you internally verify samples with this assay to determine if the buffer supplement is required.*

Brilliance *Listeria* agar plates

Agar *Listeria* plate according to Ottaviani & Agosti.

***Note**: For an NF-Validation certified method, please note that for the preparations of master solutions, you must follow the instructions from the EN ISO 6887 standard.*

STORAGE AND SHELF LIFE

- Reagents and PCR tubes with tablets should be kept refrigerated at 2–8°C. Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- After protease and Lysing Agent 2 have been added to the lysis buffer, shelf life of the solution is 1 week when stored at 2-8°C.
- If storing PCR tubes with tablets in an open kit for more than 3 weeks, seal the Mylar bag of PCR tubes into a larger bag with desiccant or store at 4ºC in a desiccation unit, if possible.
- Prepared 24 LEB Complete may be stored at 2-8°C in the dark for up to 2 weeks.

PRECAUTIONS

The BAX® System method includes sample enrichment procedures that nourish the growth of potential pathogens to detectable levels. Because pathogens can cause human illness, appropriate safety precautions must be taken when handling samples, media, reagents, glassware and other supplies and equipment that could be contaminated with potentially pathogenic bacteria.

Reagents used with the BAX® System X5 PCR assays should pose no hazards when used as directed. Before using this assay, please review the Safety Data Sheets (SDS) included with your BAX® System X5 purchase and also available at www.hygiena.com. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

The BAX® System X5 PCR Assay for *L. monocytogenes* is designed for use only with the BAX® System X5 instrument. Do not use this assay with the BAX® System Q7 instrument or other PCR platforms.

BAX® System PCR assays should always be run using the most current BAX® System X5 software version for optimal performance.

ENRICHMENT PROTOCOL

1. **Prepare Enrichment Broth**
   Prepare enrichment broth according to the manufacturer's instructions.

2. **Collect and Enrich Samples**
   Method Approved by AOAC

- *Ground beef, ground pork, ground chicken, ground turkey:* **Demi-Fraser broth:** Homogenize 25 g sample with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours. **UVM broth:** Homogenize 25 g sample with 225 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 20-24 hours.

  For both enrichments, transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Frankfurters*: **For 25 g** - Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours. **For 125 g** - Homogenize 125 g samples with 1.125L of prewarmed (30°C) UVM media. Incubate at 30°C for 23-26 hours. For both samples sizes, transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Pepperoni*: Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Fish sticks, surimi, langostinos, smoked salmon:* Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Smoked salmon:* Homogenize 25 g samples with 225 mL pre-warmed (35°C) Universal Pre-enrichment Broth. Incubate at 35°C or 22-26 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Ice cream, milk (2%) yogurt, soft cheese:* Homogenize 25 g sample with 225 mL pre-warmed (30°C) Complete Selective Enrichment Broth. Incubate at 30°C for 22-26 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Cabbage slaw, peas, spinach, strawberries, apple juice and orange juice, queso fresco cheese*: Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered *Listeria* Enrichment Broth without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Environmental sponges:* Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with D/E Neutralizing Broth. Homogenize sponge with 225

mL pre-warmed (30°C) UVM. Incubate at 30°C for 20-26 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.

Method Approved by AFNOR Certification

Test portions weighing more than 25 g have not been tested in the context of NF VALIDATION

For preparation of initial suspensions, follow instructions of EN ISO 6579 and EN ISO 6887 standards.

- Meat, seafood, dairy, vegetables, environmental samples (except charcuteries and smoked fish): Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
- Smoked fish and charcuteries: Homogenize 25 g sample with 225 mL of room temperature 24 LEB Complete media with buffer supplement. Incubate at 37°C for 24-28 hours.

TEST PROTOCOL

3. Prepare Lysis Reagent

To create a full bottle of BAX® System lysis reagent. (prepares about 60 samples)

3.1 Add 150 µL of protease and 200 µL Lysing Agent 2 to one 12 mL bottle of lysis buffer.

Note: To create smaller volumes of BAX® System lysis reagent (prepares about 4 samples), add 12.5 µL protease and 16.5 µL Lysing Agent 2 to 1 mL of lysis buffer in a separate sterile container.

4. Perform Lysis

4.1 Open the BAX® System X5 software and create a rack file (See BAX® System X5 User Guide for details).

4.2 Turn on the Automated Thermal Block and select the RT Listeria program.

***Note**: Lysis may also be performed using separate analog heating and cooling blocks. See the BAX® System X5 User Guide for details and protocols.*

4.3 Break cluster tubes apart.

4.4 Label and arrange cluster tubes in rack according to the rack file.

4.5 Transfer 200 µL prepared lysis reagent to each cluster tube.

4.6 Transfer 5 µL enriched sample to the corresponding cluster tubes.

4.7 After all transfers have been completed, secure the caps.

4.8 At the "Load Samples" prompt on the Automated Thermal Block, place the samples in the cluster tube rack onto the Automated Thermal Block.

4.9 Press the SELECT/CONTINUE button to begin automated lysis.

5. Hydrate PCR Tablets

5.1 Place a 32-well PCR tube holder onto a chilled (2-8°C) PCR cooling block.

5.2 Arrange strips of PCR tubes according to your rack file.

5.3 Remove the aluminum block with the cluster tubes of sample lysate from the Automated Thermal Block.

5.4 Press the SELECT/CONTINUE button on the Automated Thermal Block to complete the program.

5.5 Remove the caps from the first strip of tubes with the decapping tool.

5.6 Transfer 50 µL lysate (step 5.3) into PCR tubes, then seal with flat optical caps.

5.7 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

6. Complete a BAX® System Process Run

6.1 From the Info Tab labeled "Instrument" in the BAX® System X5 software window, click the RUN button to begin pre-heating the instrument.

6.2 Remove the PCR tube holder with PCR tubes from the cooling block and **visually check PCR tubes to ensure the liquid contains no air bubbles and tubes are clean.**

***IMPORTANT:** If air bubbles are present in the liquid, gently tap or flick the tubes to allow all air bubbles to escape.*

If the outside of the PCR tubes has dust, oils or residue, clean the outside of each PCR tube with a clean, lint-free lab wipe.

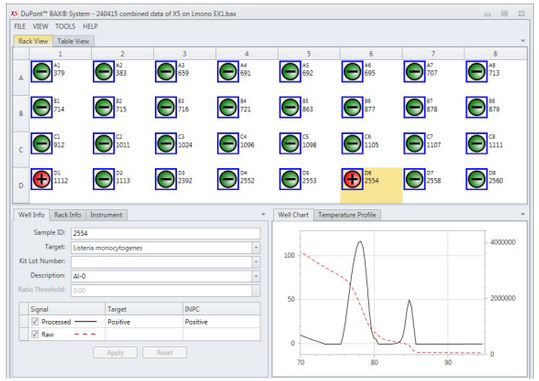
6.3 At the "Ready to load samples" prompt, open the instrument lid and load the PCR tubes into the instrument mount according to your rack file.

***IMPORTANT:** To process fewer than 32 samples, load samples first in Row A and Row D, starting at the corners. Once the two outer rows are full, then use Row B and Row C to load samples in a symmetric pattern to ensure the instrument mount remains balanced.*

6.4 Close the instrument lid as soon as possible to prevent the instrument from cooling. The process run begins automatically.

7. Review Results

Qualitative results are displayed as a grid of color-cued icons in the top half of the screen:



Green (-)	=	Negative for target organism
Red (+)	=	Positive for target organism
Yellow (?)	=	Indeterminate result*
Yellow (?) with red slash	=	Signal error*

*Refer to the troubleshooting section in the BAX® System X5 User Guide for assistance.

CONFIRMATION

Method Approved by AOAC

If desired, BAX® System results can be confirmed from the reference culture method appropriate for the sample type, such as:

- U.S. FDA Bacteriological Analytical Manual (BAM)
- USDA FSIS Microbiology Laboratory Guidebook (MLG)
- Health Canada Compendium of Analytical Methods
- International Organization for Standardization (ISO)

Method Approved by AFNOR Certification

All samples identified as positive by the BAX® System method must be confirmed in one of the following ways:

1. Using the conventional testing methods described by CEN or ISO, including purification step, if required.
2. Streak 10 µL of 24 LEB Complete enrichment onto *Brilliance*™ Listeria Agar plates and incubate at 37°C for 24-48 hours or streak 100 µL of 24 LEB Complete enrichment onto Agar *Listeria* plate according to Ottaviani and Agosti (O&A) and incubate at 37°C for 24-48 hours. With these agar formulations, *Listeria* sp. colonies exhibit a blue/green color, and *Listeria monocytogenes* colonies exhibit a blue/green color surrounded by an opaque halo. The presence of characteristic colonies is sufficient to confirm the presence of *Listeria monocytogenes*.

In the event of discordant results (presumptive positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

During the AFNOR Certification study, some samples were confirmed by subculturing in Fraser broth, incubated 24 hours at 37±1°C and streaking onto agar plates such as O&A, Rapid'*L.Mono* or PALCAM plates.

DISPOSAL

Decontaminate materials and dispose of biohazardous waste according to your site practices and as required by federal, state and local regulations.

VALIDATION

The BAX® System X5 PCR Assay for *L. monocytogenes* has been certified by the AOAC Research Institute as Performance Test Method℠ #070202. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies for foods demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture-based methods.

The BAX® System X5 PCR Assay for *L. monocytogenes* has been certified #QUA 18/05-07/08 according to NF VALIDATION rules for the detection of *Listeria monocytogenes*.
Validation studies conducted according to EN ISO 16140-2 standard and in comparison to the reference method ISO 11290-1, found this test kit's performance to satisfy the NF VALIDATION rules for all human food products and environmental samples (excluding environmental samples from primary production).
The version of software used during the NF Validation study is indicated in the NF VALIDATION certificate of the alternative method. For more information, including validity dates, please refer to certificate QUA 18/05-07/08 available at http://nf-validation.afnor.org.

TECHNICAL ASSISTANCE

For questions or comments, please contact your Hygiena representative or Diagnostics Support at 800-863-6842 in the U.S., 1-302-695-5300 outside the U.S., or email diagnostics.support@hygiena.com.

LIMITATION OF WARRANTY AND LIABILITY

NOTICE: READ THIS LIMITATION OF WARRANTY AND LIABILITY BEFORE USING THE BAX® SYSTEM EQUIPMENT, ASSAYS, AND/OR MEDIA ("BAX® SYSTEM"). If the terms are not acceptable, notify Hygiena immediately and arrangements will be made for return of the unused Equipment, assays, and/or media to Hygiena and for the refund of the purchase price, less shipping costs. USE OF BAX® SYSTEM EQUIPMENT, ASSAYS AND/OR MEDIA CONSTITUTES AN ACCEPTANCE OF ALL TERMS AND

CONDITIONS OF THIS LIMITATION OF WARRANTY AND LIABILITY. Any additional or different terms in Buyer's purchase form(s) are material alterations and hereby rejected.

1. BAX® System X5 Equipment should only be used with BAX® System X5 assays.

2. When used with BAX® System X5 PCR assays, BAX® System X5 Equipment is warranted be free of defects in materials, workmanship and design that may appear under normal and proper use within twelve (12) months from the installation date to the first end user. BAX® System X5 PCR assays are warranted to conform to the assay description under the conditions of use specified in the user documentation to the expiration date stamped on the label. BAX® System media is warranted to meet standard specifications in effect on the date of shipment. Hygiena MAKES NO OTHER WARRANTY, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING. User assumes all risk and liability resulting from use of the BAX® System X5 Equipment, PCR assays and media, whether used singly or in combination with other products.

3. BAX® System software: Hygiena warrants that for a period of 60 days from the date of first date of use by the Customer/end user, BAX® System software media will be free from defect in materials and workmanship and that the BAX® System software will substantially perform in accordance with the accompanying BAX® System software documentation. EXCEPT FOR THE EXPRESS WARRANTY ABOVE, HYGIENA MAKES NO OTHER WARRANTY, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING. User assumes all risk and liability resulting from use of the BAX® System software, whether used singly or in combination with other products.

4. The accuracy of the BAX® System can be affected by factors over which Hygiena has no control, including, without limitation, the use of the Equipment, assays and/or media in a manner that is contrary to the conditions of use, the procedures or the instructions specified by Hygiena. Because of the large number of factors over which Hygiena has no control, Hygiena makes no promise or guarantee of the accuracy of or results obtained from the use of the BAX® System. In particular, Hygiena disclaims any warranty or liability and assumes no responsibility whatever for the failure of the BAX® System due, in whole or in part, to user's failure to: (a) properly maintain Equipment, (b) maintain specified operating or storage conditions, (c) follow the specified instructions, or (d) use the proper microbiological techniques consistent with the standard of care accepted in the industry for the proper collection, storage, handling and preparation of the sample.

5. Externally caused failures, such as improper sample preparation, improper storage or loading of reagents, electrical outages, or out-of-specification environmental conditions are not covered under this warranty. Equipment failures caused by spills, abuse, misuse, negligence, or improper operation are not covered by this warranty. Modifications, service or repairs by parties other than Hygiena-authorized providers are not covered by this warranty and, in fact, void this warranty. Circumstances beyond the reasonable control of Hygiena, including fire, explosions, accidents, flood, labor trouble or shortage, war, act of or authorized by any government, inability to obtain suitable material, Equipment, fuel, power or transportation, or acts of God are not covered under this warranty.

6. The BAX® System is designed to test only for the presence of the target organisms specified in the particular assay. The BAX® System has been tested against many, but not all, strains of the target within the sample types specified in the user documentation. Hygiena, therefore, cannot and does not make any representation or warranty that the BAX® System is capable of detecting every organism in the target genus, serotype, or species in any sample source. Accordingly, the BAX® System should not be used as the sole test for the release of user's products, nor should it be used as the sole basis for determining the safety of user's products.

7. CUSTOMER/USER ASSUMES ALL RISKS IN USING THE BAX® SYSTEM AND HYGIENA OR ITS AFFILIATES, DISTRIBUTORS, ITS LICENSORS OR REPRESENTATIVES SHALL HAVE NO LIABILITY TO CUSTOMER/USER OR TO ANY OTHER PERSON OR ENTITY FOR ANY INDIRECT, INCIDENTAL, SPECIAL, PUNITIVE, EXEMPLARY OR CONSEQUENTIAL DAMAGES WHATSOEVER, INCLUDING, BUT NOT LIMITED TO, LOSS OF REVENUE OR PROFIT, LOST OR DAMAGED DATA OR OTHER COMMERCIAL OR ECONOMIC LOSS EVEN IF CAUSED BY THE NEGLIGENCE OF HYGIENA OR ITS REPRESENTATIVES AND/OR IF HYGIENA HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND/OR IF THEY ARE FORESEEABLE.

8. THE SOLE AND EXCLUSIVE REMEDY OF CUSTOMER/USER, AND THE SOLE AND EXCLUSIVE LIABILITY OF HYGIENA, ITS AFFILIATES, DISTRIBUTORS, LICENSORS OR REPRESENTATIVES FOR ANY AND ALL CLAIMS, INCLUDING BREACH OF WARRANTY, TORT, CONTRACT, STRICT LIABILITY, NEGLIGENCE OR OTHERWISE SHALL BE LIMITED TO THE FOLLOWING: (a) Should Equipment fail to conform with the Paragraph 2 warranty, Hygiena shall, at its option: repair or replace the non-conforming Equipment with new or refurbished (repaired or rebuilt) functionally equivalent Equipment or refund the purchase price; (b) Should BAX® System software fail to conform with the Paragraph 3 warranty, Hygiena will replace it free of charge; (c) For all other claims, Hygiena may, at its option, refund the purchase price or replace the Equipment, assays or media; (d) In all cases, user is responsible for the repackaging and return of non-conforming Equipment, along with the reinstallation of new or refurbished Equipment; and (e) Equipment, assays or media shall not be returned without prior written permission from Hygiena, and then only in the manner prescribed by Hygiena. The maximum liability of Hygiena, its affiliates, distributors and licensors, and whether or not based on negligence, shall not exceed in the aggregate the amount equal to: (a) the purchase price of the BAX® System X5 Equipment, PCR assays or media for which damages are claimed, or (b) in the case of BAX® System software, the amount paid for the software (if licensed separately) or twenty-five thousand dollars ($25,000.00USD). Customer/user shall notify Hygiena of any claim within thirty (30) days thereof and shall commence any action against Hygiena within one (1) year of the cause of action or otherwise be barred from any remedy. Hygiena shall not be responsible for cost, loss or liability that arise from customer/user's operation of its business, and customer/user agrees to indemnify, defend and hold Hygiena and its representatives harmless from such cost, loss or liability.