hygiena

BAX® System Real-Time PCR Assay

L. monocytogenes

Part KIT2005 (D15134303)

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 121402

KIT CONTENTS

96 PCR tubes with tablets (2 bags of 6 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 of Lysing agent 2 (1.1 mL)

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

QUA 18/10-01/19
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

INTENDED USE

Food processors and associated laboratories can use the BAX® System as a quick and reliable method for detecting *Listeria monocytogenes* in a variety of foods and environmental surfaces. This real-time PCR 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 70 minutes in the BAX® System Q7 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 User Guide for an overview of how the BAX® System method uses automated, real-time Polymerase Chain Reaction (PCR) technology.

MATERIALS

BAX® System Real-Time PCR Assay for *L. monocytogenes* (Part KIT2005 [D15134303])

BAX® System start-up package (equipment and supplies for up to 192 tests)

- BAX® System Q7 cycler/detector and computer workstation
- Heating blocks with inserts* capable of maintaining 55±2ºC and 95±3ºC
- Cooling blocks with inserts*
- PCR tube holder
- Capping/decapping tools
- Adjustable mechanical 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

**The Automated Thermal Block (Catalog No. MCH2023 [D14614252]) may be used in place of heating and cooling blocks.*

Stomacher with bags

Incubator capable of maintaining directed enrichment temperatures within ±2°C

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

Enrichment media (See BAX® System User Guide for details)
***Note**: For an NF-Validation method, please note that for the preparations of master solutions, you must follow the instructions from the EN ISO 6887 standards.*

Ottaviani & Agosti agar plates (O&A)

For smoked fish, raw and cooked delicatessen products, the buffer supplement Oxoid BO1204M/BO1204E must be added to the 24 LEB broth.

***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 AOAC, it is strongly recommended that you internally validate samples with this assay to determine if the buffer supplement is required.*

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.
- Prepared 24 LEB Complete may be stored at 2-8°C in the dark for up to 2 weeks.
- Prepared Actero™ *Listeria* Enrichment Media may be stored at 2-8°C for up to 45 days.
- 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.

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 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 purchase and also available at www.hygiena.com. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

Before using this assay for the first time, install the most current version of the BAX® System software, then run a calibration report to check that "Real Time *Listeria monocytogenes*" appears in the list of calibration files. See "Troubleshooting Calibration" in the BAX® System User Guide for details.

If the report list does not contain "Real Time *Listeria monocytogenes"*, you must recalibrate the Q7 instrument to load the required dyes. Be sure to allow enough time to complete the calibration (about 1.5 to 2 hours) before starting the assay. For instructions and tips on calibrating the instrument, see the BAX® System User Guide.

ENRICHMENT PROTOCOL – 24 LEB COMPLETE

1. Prepare enrichment broth

Prepare enrichment broth according to the manufacturer's instructions. See the BAX® System User Guide for common enrichment media recipes.

2. Collect and enrich samples

Method Approved by AOAC

- *Frankfurters*: Homogenize 125 g sample with 500 mL pre-warmed (35°C) prepared 24 LEB Complete. Incubate at 35°C for 28-48 hours.
- *Spinach, shrimp and queso fresco*: Homogenize 25 g sample with 225 mL room-temperature or pre-warmed (20-35°C) prepared 24 LEB Complete. Incubate at 35°C for 24-48 hours (for spinach), 48 hours (for shrimp), or 26-48 hours (for queso fresco).
- *Environmental surfaces (10 x 10 cm square)*: Homogenize sponge with 90 mL room-temperature or pre-warmed (20-35°C) prepared 24 LEB Complete. Incubate at 35°C for 24-48 hours.

ENRICHMENT PROTOCOL – ACTERO™ *LISTERIA* ENRICHMENT MEDIA

1. Prepare enrichment broth

Prepare Actero™ *Listeria* Enrichment Media according to the manufacturer's instructions.

2. Collect and enrich samples

- *Frankfurters*: Homogenize 125 g sample with 750 mL pre-warmed (35°C) prepared Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 26-28 hours.
- *Spinach, shrimp, smoked salmon and queso fresco*: Homogenize 25 g sample with 150 mL pre-warmed (35°C) prepared Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 22-24 hours.
- *Environmental surfaces (10 x 10 cm square)*: Homogenize sponge with 90 mL pre-warmed (35°C) prepared Actero™ *Listeria* Enrichment Media. Incubate at 35°C for 20-24 hours.

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ENRICHMENT PROTOCOL – REFERENCE METHOD MEDIA

1. Prepare enrichment broth

Prepare standard reference method enrichment broth according to the manufacturer's instructions.

2. Collect and enrich samples

- *Frankfurters*: Homogenize 125 g sample with 1125 mL pre-warmed (30°C) UVM. Incubate at 30°C for 23-26 hours. Transfer 0.1 mL primary enrichment into 10 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- *Spinach, shrimp and queso fresco*: Homogenize 25 g sample with 225 mL pre-warmed (30°C) BLEB without supplement. Incubate at 30°C for 4 hours, then add BLEB supplement. Incubate at 30°C for an additional 44-48 hours.
- *Environmental surfaces (10 x 10 cm square)*: Homogenize sponge with 225 mL pre-warmed (30°C) UVM. Incubate at 30°C for 20-26 hours. Transfer 0.1 mL primary enrichment into 10 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.

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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 6887 standard.

- *General Protocol (For all matrices excluding smoked fish, raw and cooked delicatessen*): Homogenize 25 g sample with either 225 mL 24 LEB Complete broth (ready to use) (BO 1205S) or 24 LEB base (CM 11017B) plus selective supplement (SR 0243E) at 37°C for 24-28 hours.
- *For smoked fish, raw and cooked delicatessen:* Homogenize 25 g sample with either 225 mL 24 LEB Complete broth (ready to use) (BO 1205S) plus a non-selective supplement (BO 1204M) or 24 LEB base (CM 11017B) plus selective supplement (SR 0243E) plus a non-selective supplement (BO 1204M) at 37°C for 24-28 hours.

TEST PROTOCOL

3. Prepare Equipment

3.1 Turn on the heating blocks to 55ºC and 95ºC*.

3.2 Make sure cooling blocks are chilled to 2-8ºC*.
**If using the Automated Thermal Block, follow the instructions in the Automated Thermal Block User Guide for running the RT Listeria program.*

3.3 Power on the Q7 instrument and launch the BAX® System application.

3.4 Create a rack file (see User Guide for details).

4. Perform Lysis

4.1 Break cluster tubes apart.

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

4.3 Prepare lysis reagent by adding 150 µL protease and 200 µL Lysing Agent 2 to one 12 mL bottle of lysis buffer.

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

4.5 Transfer 5 µL enriched sample to the corresponding cluster tube.
***Note**: Enrichments can be stored at room temperature until test results have been reviewed and accepted (up to 4 hours unless otherwise validated internally).*

4.6 Heat at 55°C for 30 minutes.

4.7 Heat at 95°C for 10 minutes.

4.8 Cool at 2-8°C for at least 5 minutes.

5. Hydrate PCR Tablets

5.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

5.2 Place a PCR tube rack onto a chilled (2-8°C) PCR cooling block.

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

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

5.5 Transfer 30 µL lysate (from step 4.8) into PCR tubes, then seal with flat optical caps.

5.6 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.
***Note**: PCR tablets must be hydrated and re-sealed after removing the caps from the PCR tubes.*
***Note**: An optional 10-30 minute hold of the hydrated PCR tablets in the cooling block is recommended for the Real-Time Listeria assays in order to be consistent with the Real-Time Salmonella procedure.*

6. Amplify and Detect

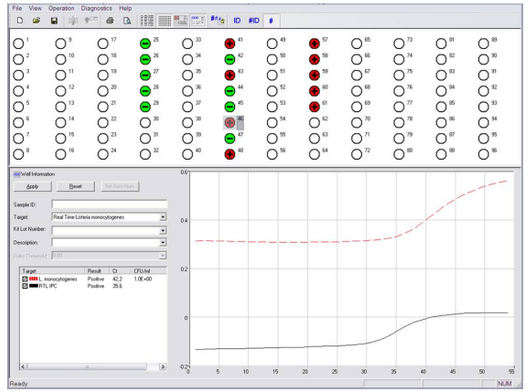
6.1 At the "Ready for Rack Load" prompt, click the NEXT button and open the instrument drawer.

6.2 Place the rack of PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.

6.3 Close the drawer, and click the NEXT button to begin automated processing.

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 User Guide for assistance.

CONFIRMATION

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If desired, BAX® System results can be confirmed from 24 LEB Complete enrichments by transferring 0.1 mL enrichment to 10 mL pre-warmed (35°C) MOPS-BLEB, incubating at 35°C for 24 hours and streaking onto MOX agar plates*. Incubate plates at 35-37°C for 24-48 hours, and confirm typical *Listeria* colonies according to the biochemical or serological methods described in the appropriate reference method.

If desired, BAX® System results can be confirmed from Actero™ *Listeria* Enrichment Media environmental sample enrichments according to the protocols described in Chapter 8 of the USDA FSIS Microbiology Laboratory Guidebook (MLG).

If desired, BAX® System results can be confirmed from reference method enrichment media by an appropriate reference method, 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)

** Brilliance™ Listeria agar plates may be used in addition to MOX agar plates for sample types with microbiologically complex enrichments.*

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

- Using the conventional testing methods described by CEN or ISO, including purification step, if required.
- Streak 0.1 mL of 24 LEB Complete enrichment onto Ottaviani and Agosti (O&A) plates and incubate at 37°C for 24-48 hours. With these agar formulations, *Listeria* spp. 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.*
- Typical colonies can be confirmed by using Bruker's MALDI Biotyper: Colonies are confirmed from O&A, PALCAM and TSYEA plates for *Listeria monocytogenes* detection. The identification of the colonies is not part of the NF Validation scope.
  NOTE: The MALDI Biotyper System Microflex LT/SH associated with the MBT 4.0 software was used. The MBT Sub-Typing module (software V4) was also used for the differentiation of Listeria species. The software first identified one Listeria species, then the sub-typing module started running automatically.

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.

DISPOSAL

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

VALIDATION

The BAX® System Real-Time PCR Assay for *L. monocytogenes* has been certified by the AOAC Research Institute as Performance Tested Method℠ #121402. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies for foods and surfaces demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture based methods.

The BAX® System Real-Time PCR Assay for *L. monocytogenes* has been certified #QUA 18/10-01/19 according to NF VALIDATION rules. Validation studies conducted according to EN ISO 16140-2 standard 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 18/10-01/19 available at http://nf-validation.afnor.org.

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. You can also call 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 Equipment should only be used with BAX® System assays.

2. When used with BAX® System assays, BAX® System 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 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 Equipment, assays and media, whether used singly or in combination with other products.

3. BAX® Software: Hygiena warrants that for a period of 60 days from the date of first date of use by the Customer/end user, BAX® software media will be free from defect in materials and workmanship and that the BAX® software will substantially perform in accordance with the accompanying BAX® 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® 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® 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, assay or media for which damages are claimed, or (b) in the case of BAX® 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.