BAX® System PCR Assay

L. monocytogenes 24E

Part KIT2002

KIT CONTENTS

96 PCR tubes with tablets (1 bag of 12 x 8 strips)
96 flat optical caps (12 x 8 strips)
Lysing agent 1 (1 x 3.0 mL bottle)
Lysing agent 2 (1 x 1.1 mL bottle)
1 bottle of protease (400 µL)
2 bottles of lysis buffer (12 mL)

QUA 18/05 -07/08
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 assay is designed to report yes/no results for *Listeria monocytogenes* at concentrations as low as 10^4 cfu/mL after 24 hours of enrichment. With a processing time of approximately 3.5 hours in the BAX® System Q7 or 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. 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, Polymerase Chain Reaction (PCR) technology.

MATERIALS

BAX® System PCR Assay for *L. monocytogenes* 24E (Part KIT2002

BAX® System Q7 start-up package (equipment and supplies for up to 192 tests) or BAX® System X5 start-up package. (equipment and supplies)

- BAX® System cycler/detector and computer workstation
- Heating blocks with inserts capable of maintaining 37±2ºC, 55±2°C and 95±3ºC* or Automated Thermal Block (Catalog No. MCH2023
- 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

**Filter tips recommended*

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)

- 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 opening and diluting, Lysing agent 1 should be stored at room temperature (20–30°C) for up to 6 months.
- After combining Lysing agent 1 and Lysing agent 2, use mixture within 4 hours.
- After protease has been added to lysis buffer, shelf life of the solution is 2 weeks 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.

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 product, please review the Safety Data Sheets (SDS) included with your BAX® System purchase and also available at www.hygiena.com. SDS for enrichment media are available at www.oxoid.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 "*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 "*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

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, spinach and shrimp:* Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
- *Queso fresco cheese:* Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 26-28 hours.
- *Stainless steel:* Homogenize sponge with 90 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 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 6579 and EN ISO 6887 standards.

- *Meat, seafood, dairy, vegetables, environmental samples (except smoked fish and charcuteries)*: 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 Equipment

3.1 Turn on heat blocks to 37°C, 55°C and 95°C*.

3.2 Make sure cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C*.

**If using the Automated Thermal Block, follow the instructions in the Thermal Block User Guide for running the 24E program (Q7) or RT Listeria program (X5).*

3.3 Power on the Q7 or X5 instrument and launch the BAX® System application.

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

4. Perform Lysis

Part One

4.1 Break cluster tubes apart.

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

4.3 Add 1.8 mL sterile distilled water to the bottle of fully thawed Lysing agent 1.

***Note**: Lysing agent 1 is solid below room temperature.*

4.4 For each sample, combine diluted Lysing agent 1 (40 µL) with Lysing agent 2 (10 µL) in 4:1 ratio.

***Note**: Prepare slightly more than required (see chart on Ready Reference for 24E PCR assays) to compensate for pipetting loss. Use within 4 hours.*

4.5 Transfer 50 µL combined lysing agents to each cluster tube.

4.6 Transfer 0.5 mL of enriched sample to the corresponding cluster tube.

4.7 Heat at 37°C for 30 minutes.

Part Two

4.8 Label and arrange a second set of cluster tubes in rack according to the rack file.

4.9 Prepare lysis reagent by adding 150 µL protease to one 12 mL bottle of lysis buffer.

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

4.11 Transfer 5 µL of heated sample from Part One (Step 4.7) to the corresponding cluster tube.

4.12 Heat at 55°C for 30 minutes.

4.13 Heat at 95°C for 10 minutes.

4.14 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 4.14) 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.

6. Amplify and Detect (For X5 Instrument loading, please reference the BAX® System X5 User's Guide)

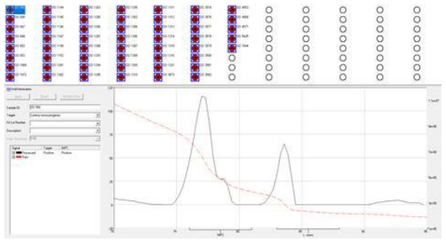
6.1 At the "Ready for Rack Load" prompt, click the NEXT button and open the instrument drawer.

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:

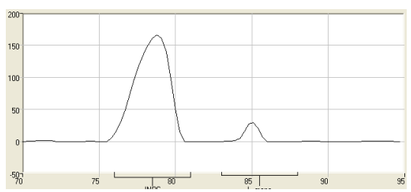


Green (-)	=	Negative for target organism
Red (+)	=	Positive for target organism
Yellow (?)	=	Indeterminate result*
Yellow (?) with red slash	=	Signal error*

*Refer to the troubleshooting section in the User Guide for assistance.

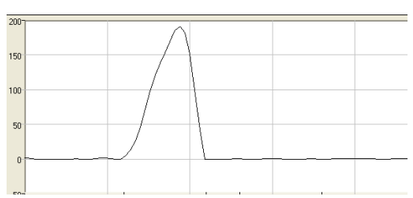
The lower pane of the well view contains a line graph of processed and/or raw data associated with the selected wells. The process data allows you to view the melting curve, which is unique to each target.

- Positive reactions for *Listeria monocytogenes* typically show one or two peaks in the positive control area (77-81°C) and one peak in the target range (83.5-86°C).



***Note**: At high target levels, the control peak may be very small or absent.*

- Negative reactions display only the positive control peak(s).



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:

- Using the conventional testing methods described by CEN or ISO, including purification step, if required.
- 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 either streaking onto a Rapid'*L.Mono* plate or subculturing in Fraser broth, incubating for 24 hours at 37±1°C and streaking onto O&A, Rapid'*L.Mono* or PALCAM plates.

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 PCR assay for *Listeria monocytogenes* 24E has been certified by the AOAC Research Institute as Performance Tested Method℠ #080901. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies on foods and surfaces demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture- based methods.

The BAX® System PCR Assay for *Listeria monocytogenes* 24E has been certified as #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 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.

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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.

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