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BAX® System PCR Assay
L. monocytogenes
Part KIT2017 (D11000157)



KIT CONTENTS

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

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. This assay is designed to report yes/no results for *Listeria monocytogenes* at concentrations as low as 10⁵ cfu/mL after enrichment. With a processing time of approximately 3.5 hours 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.

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* (Part KIT2017 [D11000157])
BAX® System start-up package (equipment and supplies for up to 192 tests)
- BAX® System cycler/detector and computer workstation
 - Heating blocks with inserts* capable of maintaining 55±2°C and 95±3°C*
 - 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

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

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 has been added to lysis buffer, shelf life of the solution is 2 weeks when stored at 2-8°C.

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

- Raw meat and poultry:** Homogenize 25 g sample with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-24 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- Processed meats:** Homogenize 25 g sample with 225 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 22-24 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- Dairy products:** Homogenize 25 g sample with 225 mL pre-warmed (30°C) Complete Selective enrichment broth. Incubate at 30°C for 22-24 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 sample with 225 mL pre-warmed (35°C) Universal pre-enrichment broth. Incubate at 35°C for 22-26 hours. Transfer 1 mL enriched sample to 9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- Other seafood, fresh vegetables, apple juice and orange juice:** 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 20 hours. Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
- Egg and egg products:** Homogenize 25 g sample with 225 mL pre-warmed (30°C) UVM 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.

TEST PROTOCOL

3. Prepare Equipment

- 3.1 Turn on heat blocks to 55°C and 95°C*.
3.2 Make sure cooling blocks are chilled to 2-8°C*.

*If using the Automated Thermal Block, follow the instructions in the Thermal Block User Guide for running the Gram Positive 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 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 of enriched sample to the corresponding cluster tube.
4.6 Heat at 55°C for 60 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 50 µL lysate (from step 4.8) into PCR tubes, then seal with flat optical caps.

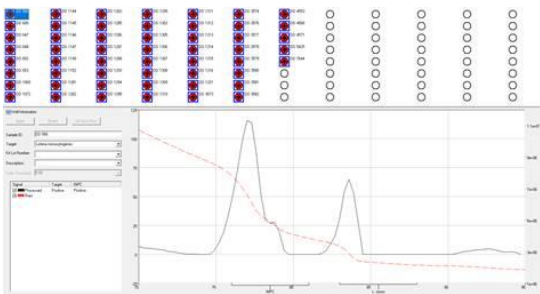
- 5.6 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

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:



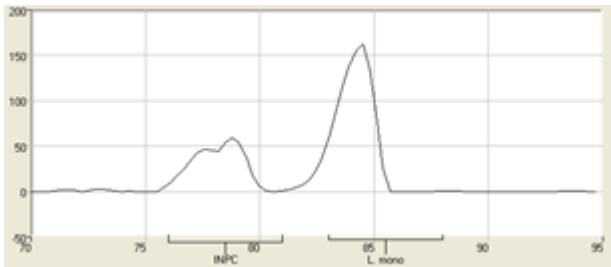
	Green (-)	=	Negative for target organism
	Red (+)	=	Positive for target organism
	Yellow (?)	=	Indeterminate result*
	Yellow (?) with red slash	=	Signal error*

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

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 (76-81°C) and one peak in the target range (83-88°C).

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



Strong *L. monocytogenes* positive with double control peak

- 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)

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* has been certified by the AOAC Research Institute as Performance Tested MethodSM #070202. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies on foods demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture based methods.

The BAX® System PCR Assay for *Listeria monocytogenes* for has been certified as an AOAC INTERNATIONAL Official Method of Analysis (OMA) #2003.12 for detecting *Listeria monocytogenes* in a variety of foods. Validation studies were performed on dairy products, fruits and vegetables (except radishes), seafood, raw and processed meats, and poultry.

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.

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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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