



BAX® System PCR Assay
Genus *Listeria* 24E
Part D13608135



KIT CONTENTS

- 96 PCR tubes with tablets (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)
- 1 package insert

INTENDED USE

Food processors and associated laboratories can use the BAX® system as a quick and reliable method for detecting *Listeria* species in food and environmental samples. The BAX® system PCR assay for Genus *Listeria* 24E detects the presence of *Listeria* species (except *L. grayi*) at concentrations as low as 10⁴ cfu/mL after 24 hours of enrichment. Results are comparable to culture method, but with quicker 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

The BAX® system uses the Polymerase Chain Reaction (PCR) to amplify specific DNA fragments, which are stable and unaffected by growth conditions. Each fragment is a genetic sequence that is unique to the targeted organism, thus providing a highly reliable indicator that the organism is present. The BAX® system simplifies the PCR process by combining the requisite PCR reagents into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After hydrating these tablets with prepared samples, the tubes remain sealed to reduce the potential for contamination.

In a typical PCR application, sample DNA is combined with DNA polymerase, nucleotides and primers that are specific for a given nucleotide sequence. The mixture then undergoes a series of

timed heating and cooling cycles. Heating denatures the DNA, separating it into single strands. As the mixture cools, the primers recognize and anneal (bind) to the targeted DNA sequence. DNA polymerase then uses nucleotides to extend the primers, thus creating two copies of the targeted fragment (amplification). Repeating cycles of denaturing, annealing and extending produces an exponential increase in the number of target DNA fragments, creating millions of copies in a very short time. If the target sequence is not present, no detectable amplification takes place.

MATERIALS

- BAX® System PCR Assay for Genus *Listeria* 24E (D13608135)
- 24 LEB Base (Oxoid CM1107 or Qualicon D13921126)
- 24 LEB Selective Supplement (Oxoid SR0243 or Qualicon D13921133)
- 24 LEB Buffer Supplement - required by AFNOR only for smoked fish and charcuteries*
 - Oxoid BO1204E or Qualicon D14327522 for 24 bottles of 10 mL each
 - Oxoid BO1204M for 10 bottles of 100 mL each

**IMPORTANT NOTE: The 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 validate samples with this assay to determine if the buffer supplement is required.*

Note: A full kit (96 tests) of food samples uses 2 bottles (1 kg) base and 5 packs (50 vials) selective supplement. A full kit of environmental samples uses 1 bottle (500 g) base and 2 packs (20 vials) selective supplement.

Included in BAX® system start-up package:

- BAX® system cyclor/detector
- Computer workstation with printer
- Heating blocks (2)
- Cooling blocks (lysis and PCR tubes)
- 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*
- Tips for all pipettes (100 µL, 250 µL, 5.0 mL)*
- Powder-free nitrile gloves*

**Supply for 96 tests included in BAX® system start-up package*

Not included in BAX® system start-up package:

- Stomacher
- Third heating block
- 2.5 mL pipette tips for dispensing lysing agents (ThermoFisher #9404173 or equivalent)
- 0.3–0.6% sodium hypochlorite bleach (10% of standard 3–6% household solution)
- Sterile water
- Standard solutions, consumables and media

STORAGE AND SHELF LIFE

Assay Reagents

Kits should be kept refrigerated at 2–8°C. *Do not freeze.*

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 mixing protease and lysis buffer, solution can be stored at 2–8°C for up to 2 weeks.

Use all other reagents in the kit by the expiration date stamped on the individual labels.

Enrichment Media

Unopened, unprepared 24 LEB base should be stored at 10–30°C out of direct light until the expiration date stamped on the label.

Unopened, unprepared 24 LEB selective supplement should be stored at 2–8°C in the dark until the expiration date stamped on the label.

Unopened 24 LEB buffer supplement should be stored at 2–8°C until the expiration date stamped on the label.

After diluting and autoclaving, 24 LEB base can be stored unopened at 2–8°C for up to 2 weeks*.

After combining 24 LEB base and selective supplement, the mixture can be stored at 2–8°C in the dark for up to 2 weeks*. Mixture must be brought to room temperature prior to use.

**For more information on prepared media stability, contact Oxoid at www.oxoid.com.*

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 Material Safety Data Sheets (MSDS) included with your BAX® system purchase and also available at www.qualicon.com. MSDS for enrichment media are available at www.oxoid.com. Refer to your site practices for safe handling of materials at extreme temperatures.

TEST PROTOCOL

1. Prepare enrichment broth

Calculate the volume of enrichment broth needed for the number and type of samples you are testing. The following preparation guidelines prepare sufficient broth for 2.2 food samples or 5.5 environmental samples.

- 1.1 Add 21.75 g 24 LEB base to 500 mL deionized water, mix and autoclave at 121°C for 15 minutes. Cool to 50°C.
- 1.2 Hydrate 1 vial of 24 LEB selective supplement with 5 mL of sterile deionized water, and mix to dissolve.

1.3 Add vial of hydrated supplement to 500 mL base and mix thoroughly. Cool to 25–30°C before use. Final pH 7.4±0.2 at 25°C.

1.4 If using 24 LEB buffer supplement, add 10 mL pre-warmed (37°C) supplement to each 225-mL volume of prepared 24 LEB and mix.

2. Collect and enrich samples

- 2.1 For **food**, homogenize 25 g sample with 225 mL room-temperature (25–30°C) prepared enrichment broth in filterless stomacher bag.
- 2.2 For **environmentals**, wipe a 4 x 4 in (10 x 10 cm) area with a sponge pre-moistened in DE neutralizing broth, add to 90 mL room-temperature (25–30°C) prepared enrichment broth and mix.

2.2 For **all** sample types, incubate at 37°C for 26±2 hours.

3. Prepare equipment

- 3.1 Make sure that both lysis and PCR cooling blocks are chilled to 2–8°C.
- 3.2 Make sure that heating blocks are at the correct temperature.
- 3.3 Power on the cyclor/detector and launch the BAX® system application.
- 3.4 Create a rack file (see *User Guide* for details) and choose “Genus *Listeria*” from the drop-down target menu.
- 3.5 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

4. Perform part one of lysis

4.1 Add 1.8 mL sterile deionized water to the bottle of fully thawed Lysing agent 1 and mix.

Note: Lysing agent 1 is solid below room temperature.

4.2 Combine lysing agents in 4:1 ratio (40 µL of diluted Lysing agent 1 to 10 µL Lysing agent 2 per sample) in a sterile tube. Prepare slightly more than required (see chart on Ready Reference for 24E PCR assays) to compensate for pipetting loss. Use within 4 hours.

4.3 Transfer 50 µL of the combined lysing agents to cluster tubes.

4.4 Without agitating the bag, remove 0.5 mL of enriched sample from the top of the bag (but below any fatty layer that may have formed) and transfer to cluster tubes.

Note: If sample enrichment includes 24 LEB buffer supplement, agitate the bag and allow particles to settle before transferring enriched sample to cluster tubes.

4.5 Cap tubes, mix and place in heating block at 37±1°C for 30 minutes.

5. Perform part two of lysis

5.1 Mix 150 µL protease with 12 mL lysis buffer.

5.2 Transfer 200 µL of the protease mix to a second set of cluster tubes.

5.3 Use the multi-channel pipette to transfer 5 µL heated enriched sample to cluster tubes with protease mix.

5.4 Cap second set of tubes and place in heating block at 55°C for 30 minutes.

5.5 Transfer tubes to 95°C heating block for 10 minutes.

5.6 Transfer tubes to cooling block (2–8°C) for 5 minutes.

6. Hydrate PCR tablets

6.1 Place PCR tube holder onto a chilled (2–8°C) PCR cooling block insert.

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

6.3 Transfer 50 µL cooled lysate to each PCR tube, and seal with flat optical caps.

7. Amplify and detect

7.1 At the “Ready for Rack Load” prompt, open the instrument drawer.

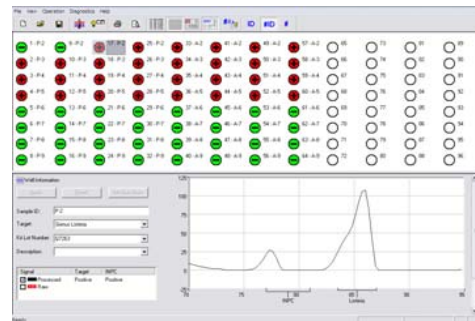
7.2 Place the rack of PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.

7.3 Close the drawer and click the NEXT button to begin automated processing. A status bar displays progress.

8. Review results

When processing is complete (about 3.5 hours), follow the screen prompts to remove your samples and review the results. Results are displayed as a grid of well icons in the top half of the screen:

- If *Listeria* are present in the sample, the well is red with a “plus” sign (positive).
- If no *Listeria* are present in the sample, the well is green with a “minus” sign (negative).
- If the target and the internal positive control (IPC) are negative, the well is yellow with a “question mark” sign (indeterminate).
- A well that is yellow and outlined in red with a red bar across the center indicates a signal error. Call Qualicon for assistance.



The lower pane of the well view contains a line graph of the melting curve for *Listeria*.

- Positive reactions typically show one peak in the positive control area (77-81°C) and one or two peaks 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

If desired, positive results can be confirmed by plating 10 µL of enriched sample onto Brilliance™ *Listeria* agar [Oxoid CM1080, SR0227E and SR0228E]. Incubate at 37°C for 24–48 hours, then test presumptive colonies with biochemical or serological methods as described in the FDA-BAM or FSIS-MLG.

DISPOSAL

Decontaminate materials and dispose of biohazardous waste according to your site practices and as required by federal, state and local regulations. If you have questions about proper waste disposal at your site for the materials provided by DuPont Qualicon, please refer to the MSDS or call for assistance.

VALIDATION

The BAX@ system PCR assay for Genus *Listeria* 24E has been certified by the AOAC Research Institute as Performance Tested MethodSM #050903. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies on frankfurters, spinach, cooked shrimp, queso fresco cheese and stainless steel demonstrated BAX@ system sensitivity and specificity equal to or better than the official FDA-BAM or USDA-FSIS culture-based methods.

The BAX@ System PCR Assay for Genus *Listeria* 24E has been certified (#QUA 18/06 – 07/08) according to AFNOR validation rules. Validation studies conducted according to ISO 16140 standards found this test kit's performance to satisfy the AFNOR certification rules for all human food products and environmental (reference method ISO 11290-1), except that smoked fish (and optionally charcuteries) requires the addition of the 24 LEB buffer supplement to the enrichment media (reference method ISO 11720-1).

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. You can also call 800-863-6842 in the U.S. or visit www.qualicon.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 DuPont Qualicon (“Qualicon”) immediately and arrangements will be made for return of the unused equipment, assays, and/or media to Qualicon 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 Qualicon 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 Qualicon. Because of the large number of factors over which Qualicon has no control, Qualicon makes no promise or guarantee of the

accuracy of or results obtained from the use of the BAX@ system. In particular, Qualicon 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 Qualicon-authorized providers are not covered by this warranty and, in fact, void this warranty. Circumstances beyond the reasonable control of Qualicon, 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. Qualicon, 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 QUALICON 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 QUALICON OR ITS REPRESENTATIVES AND/OR IF QUALICON HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND/OR IF THEY ARE FORESEEABLE.

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