



BAX® System Real-Time PCR Assay

Campylobacter jejuni/coli/lari

Part KIT2018

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 *Campylobacter* in a variety of foods. This real-time PCR assay was designed to report yes/no results along with quantitative values* for three species, *C. jejuni*, *C. coli* and *C. lari* at concentrations as low as 10^4 CFU/mL, with or without 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 practices, including the safe handling and disposal of potentially pathogenic materials.

*AOAC-RI has granted PTM certification to this assay only for qualitative results on enriched samples.

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 US or non-US 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 *Campylobacter jejuni/coli/lari* (Part KIT2018)

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 37 ± 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) can be used in place of heating and cooling blocks.

**Filter tips recommended.

Stomacher with bags

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

Apparatus for microaerobic gassing (5% O₂, 10% CO₂, 85% N₂)

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

STORAGE AND SHELF LIFE

- Reagent packages 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 the 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. 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 *Campylobacter*" 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 *Campylobacter*", 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

- *Poultry carcass rinses*: Homogenize sample 1:1 with pre-warmed (42 °C) double-strength Bolton broth with supplement (no blood). The volume prepared should be sufficient to allow < 2.5 mm headspace from the top of an air-tight tube or other air-tight container. Incubate at 42 °C for 24-48 hours.
- *Processed turkey*: Homogenize 25 g sample with 225 mL pre-warmed (42 °C) single-strength Bolton broth with supplement (no blood). Incubate under microaerophilic* conditions at 42 °C for 24-48 hours.

*Note: *Campylobacter* are microaerophilic and therefore susceptible to environmental stresses, such as exposure to air, drying, low pH and prolonged storage. Sample preparation usually requires a gassing step (5% O₂, 10% CO₂, 85% N₂) and incubation in a microaerophilic atmosphere.

DIRECT (NON-ENRICHED) PROTOCOL

Some sample types, such as chicken carcass rinses, are expected to have high concentrations of *Campylobacter*. When low-level detection is not a concern, the enrichment step can be omitted.

TEST PROTOCOL

3. Prepare Equipment

3.1 Turn on the heating blocks to 37 °C and 95 °C*.

3.2 Make sure cooling blocks are chilled at 2-8 °C*.

*If using the Automated Thermal Block, follow the instructions in the Thermal Block User Guide for running the Gram-Negative 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 lysis reagent to each cluster tube.

4.5 Transfer 5 µL enriched sample to the corresponding cluster tube.

4.6 Heat at 37 °C for 20 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.

6. Amplify and Detect

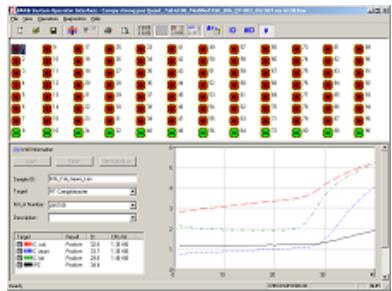
6.1 At the "Ready for Rack Load" prompt, click the NEXT button and open the instrument drawer.

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.

Quantitative results are displayed in the bottom half of the screen when you click on a well:

Target	Result	Ct	CFU/ml
<input checked="" type="checkbox"/> C. coli	Positive	32.8	1.3E+06
<input checked="" type="checkbox"/> C. jejuni	Positive	33.7	1.3E+06
<input checked="" type="checkbox"/> C. lari	Positive	29.8	1.4E+06
<input checked="" type="checkbox"/> IPC	Positive	34.4	

- For each differentiated target, positive/negative results are reported, along with the cycle number at which the fluorescent signal reached the detection threshold (Ct).
- The last column provides a value for the cell concentration that was present in the sample going into lysis. For non-enriched samples, this value is a good measure of raw contamination levels; otherwise, this value reflects the concentration level after enrichment.

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:

- US 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 Real-Time PCR Assay for *Campylobacter jejuni/coli/lari* has been certified by the AOAC Research Institute as Performance Test MethodSM #040702. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies for enriched foods demonstrated BAX System sensitivity and specificity equal to or better than the reference culture-based methods.

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. You can also call 800-863-6842 in the US, 1-302-695-5300 outside the US, or email diagnostics.support@hygiena.com www.hygiena.com/support

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