



BAX® System Real-Time PCR Assay

Campylobacter jejuni/coli/lari Part KIT2018 (D12683449)

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 10⁴ 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 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 *Campylobacter jejuni/coli/lari* (Part KIT2018 [D12683449])

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 [D14614252]) 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). Volume prepared should be sufficient to allow < 2.5 mm headspace from the top of 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 microaerobic* 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 microaerobic 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.

Method Approved by NordVal

- *Cloacae Swabs (>100 cfu/mL):* After collection of cloacae swab, place swab into vial containing 2 mL of 0.9% sodium chloride and 0.1% peptone. Proceed to BAX® lysis procedure.

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

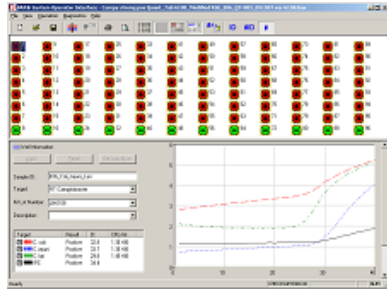
- 5.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.
- 5.2 Place a PCR tube rack onto a chilled (2-8°C) PCR cooling block.
- 5.3 Arrange strips of PCR tubes according to your rack file.
- 5.4 Remove the caps from the first strip of tubes with the decapping tool.
- 5.5 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:

- 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 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.

The BAX@ System Real-Time PCR Assay for *Campylobacter jejuni/coli/lari* has been certified by NordVal International as fulfilling the requirements of the NordVal Validation Protocol (NordVal #039). This test kit's performance was reviewed by NordVal International and was found to perform to the manufacturer's specifications for direct testing of cloacae swabs (above 100 cfu/g). Validation studies for the swabs demonstrated BAX@ System sensitivity and specificity equal 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 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.

- BAX@ System Equipment should only be used with BAX@ System assays.
- When used with BAX@ System assays, BAX@ System Equipment is warranted to 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,

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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, species or serotype 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.

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