



BAX® System PCR Assay for *Cronobacter (E. sakazakii)* Part KIT2001 (D11801836)

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 ES lysis buffer (12 mL)

INTENDED USE

Food processors and associated laboratories can use the BAX® System as a quick and reliable method for detecting *Enterobacter sakazakii* (now recognized as *Cronobacter*) in selected foods and environmental surfaces. This assay is designed to report yes/no results for *Cronobacter* 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.

Note: Please refer to your accreditation agency for any specific requirements.

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, Polymerase Chain Reaction (PCR) technology.

MATERIALS

BAX® System PCR Assay for *Cronobacter* (Part KIT2001 [D11801836])

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

- BAX® System cycler/detector and computer work station
- 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]) may be used in place of heating and cooling blocks.*

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)

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 assay, 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 “*Cronobacter*” 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 “*Cronobacter*”, 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 that BAX® System User Guide for common enrichment media recipes.

2. Collect and Enrich Samples

Follow the enrichment protocols for the selected sample types as described in the most current version of the BAX® System User Guide.

TEST PROTOCOL

3. Prepare Equipment

3.1 Turn on the heating blocks for 37°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 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 ES 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 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

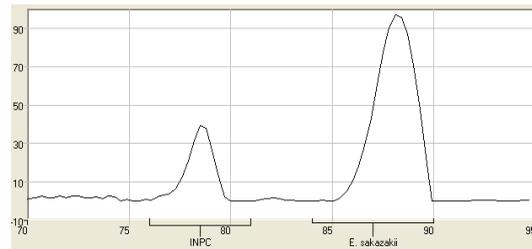
Qualitative results are displayed as a grid of color-coded icons in the top half of the screen:

	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.

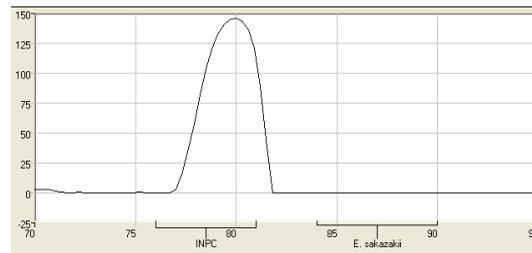
The lower pane of the well view contains a line graph of processed and/or raw data associated with the selected wells. The process data allows you to view the melting curve, which is unique to each target.

- Positive reactions for *Cronobacter* show one peak in the positive control area (76-81°C) and one peak in the target range (84-90°C).



Strong *Cronobacter* positive

- Negative reactions display only the positive control peak.



- 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 *Cronobacter* has been internally validated on selected foods and environmental surfaces. The results of these studies demonstrate sensitivity and specificity equivalent to the reference culture based methods for detecting *Cronobacter*.

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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