

BAX[®] System X5

User Guide

BAX[®] System X5



© Copyright 2021 Qualicon Diagnostics LLC, a Hygiena™ Company or its affiliates. All rights reserved worldwide. No part of this publication may be reproduced or transmitted in any form, or by any information storage and retrieval system, without the prior agreement and written permission of Hygiena.

Information in this document is subject to change without notice. Hygiena assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Hygiena be liable for incidental, special, multiple, or consequential damages in connection with or arising from the use of this document.

Part Number: UG-002 Rev 04 v1.3
Effective Date: 02/01/2021

TRADEMARKS

The Hygiena™ logo and BAX® are trademarks or registered trademarks of Hygiena or its affiliates. SYBR is a registered trademark of Thermo Fisher Scientific or its subsidiaries in the U.S. and/or certain other countries. MATLAB is a registered trademark of The Mathworks, Inc. Microsoft Windows® is a registered trademark of Microsoft Corporation. All other trademarks are property of their holders.

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. Read the Limitation of Warranty and Liability before using product.

LICENSING

MATHWORKS

MATLAB 1984-2005 The MathWorks, Inc. is deployed with the BAX® System application and governed by a certain license agreement between Hygiena and MathWorks. License agreement files (MathWorks or Third-Party) that are included with the MCR Libraries ("MCR Library License") may not be modified or removed.

THERMO FISHER SCIENTIFIC

The SYBR® Green I stain in some BAX® System PCR assays is sold under license from Life Technologies Corporation, a Thermo Fisher Scientific Company, for use only in homogenous polymerase chain reaction assays in combination with the DNA detection technology of Hygiena, and is covered by pending and issued patents.

PROMEGA CORPORATION

GoTaq® Hot Start Polymerase in some BAX® System PCR assays is sold under distribution agreement with Promega Corporation. GoTaq® Hot Start Polymerase is manufactured by and licensed to Promega Corporation under U.S. Patent Nos. 5,338,671 and 5,587,287 and their corresponding foreign patents and sold to Hygiena for distribution.

Contents

Introduction and Overview	4
About the BAX® System	4
User Assumptions.....	5
Text Conventions	5
Ordering Information	6
Other Required Equipment and Supplies.....	7
Storage and Disposal Information	8
Shipping Instructions.....	8
Technical Assistance	8
I. The BAX® System X5 Instrument	9
Instrument Specification	10
Environmental Parameters.....	11
Instrument Components	12
Safety Precautions	14
Instrument Installation.....	17
Instrument Configuration.....	18
Instrument Maintenance	20
Instrument Decontamination	21
Capping/De-Capping Tools	22
II. The BAX® System X5 Software	23
The BAX® System X5 Software Window	24
Creating a Rack File.....	27
Printing Reports	28
Installing and Upgrading the BAX® System Software.....	30
III. The BAX® System X5 PCR Assays.....	33
Using the <i>E. coli</i> O157:H7 Assay	35
Using the Genus <i>Listeria</i> Assay	44
Using the <i>L. monocytogenes</i> Assay.....	54
Using the <i>Salmonella</i> Assay	63
Reviewing BAX® System Results	74
Reviewing Results Graphs Well Charts.....	75
Best Practices for Sample Enrichment, Preparation and Storage.....	76
Using Analog Heating and Cooling Blocks with the BAX® System Assays	80
Enrichment Media Recipes	82
IV. Troubleshooting the BAX® System	93
V. BAX® System X5 Limitation of Warranty & Liability	99

About the BAX® System

The BAX® System is an automated molecular detection method for identifying foodborne pathogens, spoilage organisms and other microbes in raw ingredients, finished products and environmental samples. For more than 20 years, food companies, service labs and government regulators around the world have relied on the BAX® System to help them get products to market faster while protecting both quality and reputation.

The BAX® System was the first molecular food testing method to use Polymerase Chain Reaction (PCR) technology, which rapidly creates millions of copies of the targeted DNA fragment, if present, in a very short time. The result is a clearly detectable “yes-or-no” answer within hours of starting the assay, without the need for expert interpretation.

The BAX® System combines the power of PCR with automated detection to significantly reduce hands-on time, minimize the potential for cross-contamination, and provide for consistent results based on computerized algorithms for analysis. Simply load your prepared samples, run the program and read the results on screen.

To further simplify the process, the BAX® System assays provide all requisite PCR primers, polymerase and nucleotides as a stable, dry, manufactured tablet in sealed PCR tubes to eliminate the need for reagent preparation. After amplification, these tubes remain sealed for the detection phase, significantly reducing the potential for sample or laboratory contamination with the amplified PCR product.

About the Polymerase Chain Reaction (PCR)

PCR is an analytical tool for quickly replicating a targeted DNA fragment. Each BAX® System assay targets specific DNA fragments unique to the organism of interest, providing a stable, reliable indicator that the organism is present and vastly reducing the potential for cross reactions with similar organisms.

In a typical application, sample DNA is combined with DNA polymerase, nucleotides and primers that are specific for a given nucleotide sequence. This mixture then goes through 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 sequence. DNA polymerase then uses the provided nucleotides to extend the primers, thus creating two copies of the targeted DNA fragment (amplification). Repeating the cycle 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.

About Automated Detection

As the target DNA is amplified through PCR in the BAX® System instrument, fluorescent dye in the BAX® System PCR tablets binds to the double-strands, causing it to emit a fluorescent signal. After amplification, the BAX® System begins a detection phase where the fluorescent signal is measured. During detection, the temperature of the samples is raised to the point where the DNA strands separate (denature), releasing the dye and lowering the signal. This change in fluorescence is plotted against temperature to generate a melting curve, which is interpreted by the BAX® System software as positive or negative results.

User Assumptions

- Users are qualified lab personnel who follow good microbiology laboratory practices, including the safe handling and disposal of potentially pathogenic materials.
- Users are familiar with Microsoft® Windows® operating system, including navigation and backup procedures.

Text Conventions

This manual uses the following text conventions to indicate software commands/selections/messages:

Menu selections appear in UPPER CASE separated by > to indicate sequence.

- Field names and screen names appear in **bold lower case**.
- Screen buttons may appear in upper case in a **BOX**
- System messages appear in “quotes.”
- *Notes appear in italics.*
- Safety symbols are detailed in Chapter I, but the main categories are as follows:



General alert or warning



Live power may still be
available even with power
off or failed fuse.



Hot surface! Avoid touching.

Ordering Information

For more information about the BAX® System and other products from Qualicon Diagnostics, contact your local representative or visit <https://www.hygiene.com/bax.html>

Note: The BAX® System X5 instrument is designed for use with BAX® System X5 assays and associated materials only. BAX® System Q7 assays cannot be used with the BAX® System X5 instrument.

BAX® System Equipment and Supplies

Part Number	Description	Contents
MCH2005	BAX® System X5 Instrument Package	<ul style="list-style-type: none"> BAX® System X5 instrument BAX® System X5 reference card Bag of empty, white PCR tubes for sample balancing (96) Power supply and cables Network cable USB memory drive with user documentation and software Flight case for transport/shipping
MCH2003	BAX® System X5 Computer Package	<ul style="list-style-type: none"> Laptop computer pre-loaded with BAX® System X5 software Computer mouse Security cable lock
MCH2000	BAX® System X5 Auxiliary Package Note: Analog heating and cooling blocks may be used in place of the Automated Thermal Block. See "Using Analog Heating and Cooling Blocks with the BAX® System Assays" for details.	<ul style="list-style-type: none"> Automated Thermal Block with metal tube holders and power supply 32-well aluminum cold block with insulator for PCR tubes 32-well sample carriers (2) Capping/decapping tools (2) Multi-channel pipette (1) Adjustable mechanical pipettes (2) Repeating pipette (1) Pipette tips with barriers Cluster tubes with caps and racks powder-free nitrile gloves

BAX® System Enrichment Media

Part Number	Description	Contents
MED2016	BAX® System MP Media StatMedia™ soluble packets	<ul style="list-style-type: none"> 20 pouches x 5 packets per pouch x 33.75 g per packet
MED2003	BAX® System MP Media - bulk powder	<ul style="list-style-type: none"> 2.5-kg tub
MED2002	BAX® System Media for <i>Listeria</i> - bulk powder	<ul style="list-style-type: none"> 2.5-kg tub

BAX® System X5 PCR Assays

Part Number	Description	Contents
KIT2022	BAX® System X5 PCR Assay for <i>E. coli</i> O157:H7	<ul style="list-style-type: none"> • Lysis buffer (2 x 12 mL) • Protease (400 µL) • PCR tubes with tablets (64) • Flat optical caps for PCR tubes (96)
KIT2024	BAX® System X5 PCR Assay for Genus <i>Listeria</i>	<ul style="list-style-type: none"> • Lysis buffer (2 x 12 mL) • Protease (400 µL) • Lysing agent 2 (1.1 mL) • PCR tubes with tablets (64) • Flat optical caps for PCR tubes (96)
KIT2023	BAX® System X5 PCR Assay for <i>L. monocytogenes</i>	<ul style="list-style-type: none"> • Lysis buffer (2 x 12 mL) • Protease (400 µL) • Lysing agent 2 (1.1 mL) • PCR tubes with tablets (64) • Flat optical caps for PCR tubes (96)
KIT2025	BAX® System X5 PCR Assay for <i>Salmonella</i>	<ul style="list-style-type: none"> • Lysis buffer (2 x 12 mL) • Protease (400 µL) • PCR tubes with tablets (64) • Flat optical caps for PCR tubes (96)

Other Required Equipment and Supplies

In addition to the BAX® System X5 instrument, start-up package, and PCR assays, the following items are required to use the BAX® System method. These items are not supplied by Hygiena.

- Stomacher capable of homogenizing samples between 200-260 rpm (or equivalent)
- Filtered stomacher bags
- Incubators capable of maintaining designated temperatures within $\pm 2^{\circ}\text{C}$
- Standard solutions, consumables and media

Storage and Disposal Information

Equipment Storage

- Clean and maintain required equipment according to the instructions of the manufacturer.
- Cooling blocks should be kept refrigerated at 2-8°C and used within 30 minutes of removal from refrigerator.

Note: Users following Health Canada's MFLP-30, use PCR cooling block which have been frozen at -20°C.

- Pipettes should be calibrated to deliver within 10% of required volumes. Barrier tips are recommended for all pipettes.

Assay Storage

- Keep BAX® System reagents and unused PCR tubes refrigerated at 2–8°C. Do not freeze.
- If storing PCR tubes with tablets in an open kit for more than 3 weeks, seal the Mylar bag of PCR tubes into a larger bag with desiccant or store at 4°C in a desiccation unit, if possible.

Reagent Storage

- 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 two weeks when stored at 2- 8°C.
- For BAX® System X5 *Listeria* assays, lysis reagent made with protease, Lysing Agent 2 and lysis buffer can be stored for up to one week at 2-8°C

Shipping Instructions

In the event your BAX® System X5 instrument requires service, contact Hygiena or your local distributor to obtain a return authorization number and instructions for returning the unit:

- Thoroughly clean the unit of any biohazardous substances.
- To avoid damage to the instrument, use ONLY the BAX® System X5 flight case for shipping the instrument.
- Ship the unit to the address on the return form. Purchasing insurance from your shipper is strongly recommended.

Technical Assistance

If you have any questions or comments on the BAX® System, contact your local distributor or Hygiena representative for technical assistance.

You can also contact Hygiena Diagnostics Support directly at diagnostics.support@hygiena.com

Hygiena Diagnostics Support

United States and Canada

Phone: (+1) 800-863-6842

Fax: (+1) 302-351-6454

Outside the United States and Canada

Phone: (+1) 302-695-5300



System X5

I. The BAX® System X5 Instrument

hygiena™

Instrument Specifications

Item	Specification
Dimensions (width × depth × height)	9.45 in × 10.63 in × 9.06 in (24 cm × 27 cm × 23 cm)
Weight	15.4 lbs (7 kg)
Power supply	100-240 VAC; 50-60 Hz
Power usage	Operates at 24 V with 6.25 A; maximum usage 170 VA
Mains power consumption	170 W
Noise level	< 40 dB (A)
Heat output during run (mean value)	~58 Btu/h or 170 kJ/h
Heat output in standby	~34 Btu/h or 36 kJ/h
Sample capacity	Up to 32 samples per run
Processing time	3.5 hours for standard PCR protocols
Fluorescence spectrometer excitation	Filtered LED, 500 nm
Fluorescence spectrometer detection	Spectrometer, 2 nm (emission wavelength)
Channels	120 optical channels
Temperature range	40-99°C
Heating rate	4°C/s
Cooling rate	3°C/s
Thermal homogeneity	±0.25°C
Thermal accuracy	±0.50°C

Environmental Parameters

Item	Specification
Minimum bench space required (width × depth × height)	9.45 in × 12.21 in × 14.96 in (24 cm × 31 cm × 38 cm)
Temperatures allowed during transportation, storage and packaging	-20 to +60°C
Relative humidity allowed during transportation, storage and packaging	10-95%, no condensation
Altitude/pressure allowed during transportation, storage and packaging	0-3,000 m above sea level / 70-106 kPa
Temperatures allowed during operation	15-32°C
Relative humidity allowed during operation	Max. 80% at 32°C, no condensation / Min. 30% at 15-32°C
Altitude/pressure allowed during operation	0-2,000 m above sea level / 80-106 kPa

Instrument Components

Instrument Lid

The lid of the BAX® System X5 instrument shows different colors based on the status of the instrument:

During a process run

Color	Instrument Status
Blue (steady)	The instrument is pre-heating for a process run.
Green (steady)	The instrument is ready for a process run. Samples are not loaded yet.
Red (pulsing)	The instrument is pre-heating during a process run.
Red and Blue (alternating)	The instrument is alternating between heating and cooling samples during a process run.
Blue (pulsing)	The instrument is acquiring optical data during a process run
Cyan (steady)	The process run is complete. Samples can be removed from the instrument.



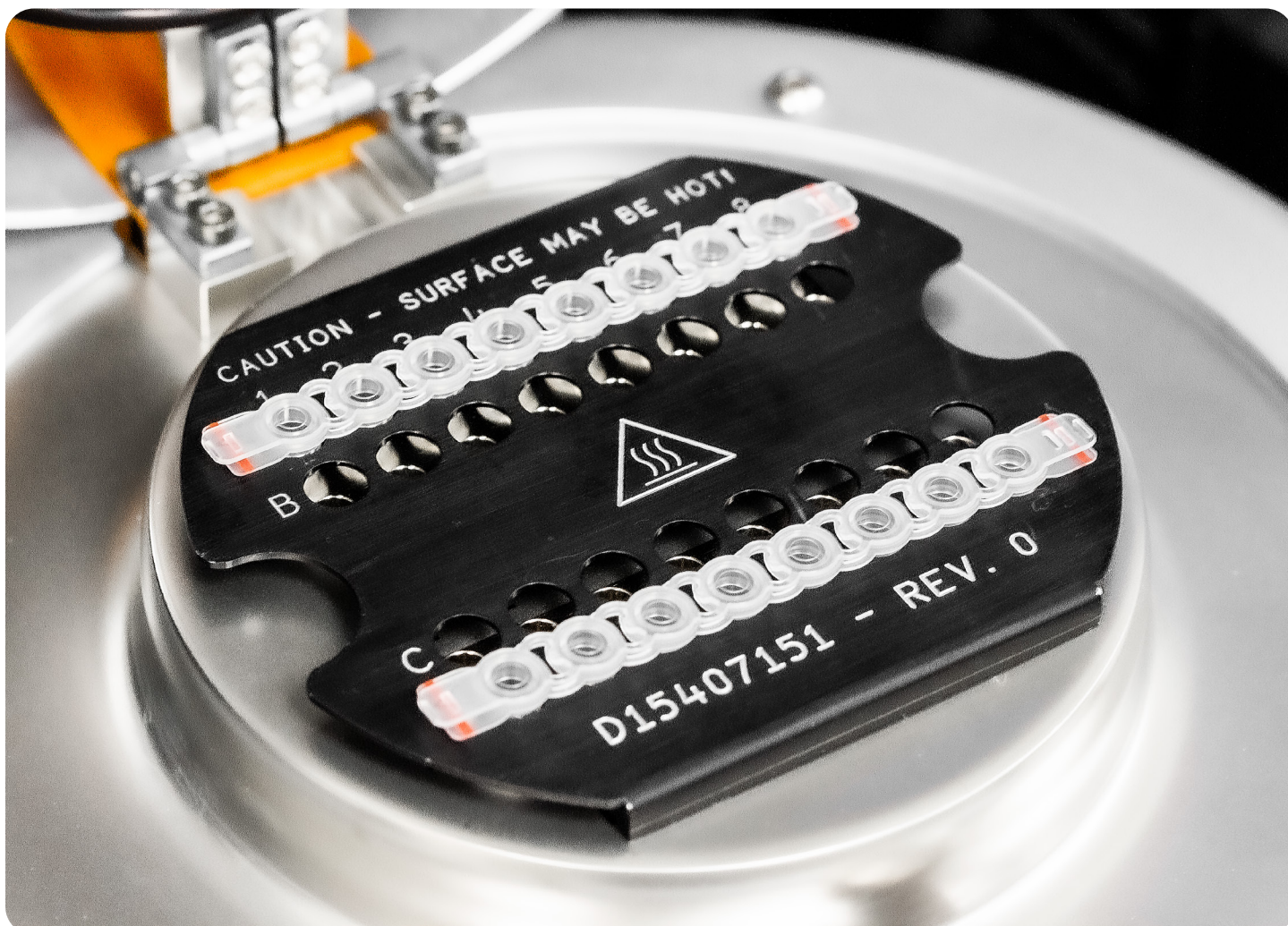
When instrument is not processing samples

Color	Instrument Status
Blue (pulsing)	The instrument is powering on.
Red (steady)	The instrument lid is open.
Red (flashing)	An instrument error is detected.

Instrument Mount

The instrument mount accepts up to four strips of eight BAX® System X5 PCR tubes each or up to 32 single tubes. The rows and columns of the instrument mount are numbered according to the display in the plate view.

If you are processing fewer than 32 samples in the BAX® System X5 instrument, it is important to load samples in a symmetric pattern to ensure the instrument is balanced and to prevent potential damage to the instrument lid. We recommend loading samples first in Row A and Row D, starting at the corners. Once the two outer rows are full, then use Row B and Row C to load samples in a symmetric pattern.



Note: BAX® System Q7 PCR tubes cannot be used with the BAX® System X5 instrument.

Safety Precautions

Instrument Safety



WARNING - PHYSICAL INJURY HAZARD. Use this instrument as specified by Hygiena to avoid personal injury or damage to the instrument



DANGER - ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility. Use only the power cables and Ethernet connector supplied with the system package.



CAUTION! Any break in the electrical ground wire, whether inside or outside the instrument, or disconnection of the electrical ground connection, could create a hazardous condition.



CAUTION! Do not under any circumstances attempt to modify or deliberately override the safety features of this system.

The BAX® System X5 instrument is for indoor use only.

Do not place the instrument in direct sunlight or close to radiators or heating devices.

Do not place the BAX® System X5 instrument next to any source of vibration or electromagnetic interference, or instrumentation that has high inductance (e.g., refrigerators, centrifuges, or mixers).

Do not place anything on top of the instrument.

Do not place anything under the instrument, such as sheets of paper, since these could block the air inlet.

Biological Safety



Samples analyzed with this instrument may contain potentially bio-hazardous material such as bacterial, fungal or viral agents. As with all potentially biohazardous specimens, universal safety precautions should be taken when handling and processing samples.



The BAX® System method includes sample preparation 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. In particular, individuals who are pregnant or immunocompromised should avoid any potential contact with *Listeria monocytogenes*




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), available on the web at www.Hygiena.com. Refer to your site practices for safe handling of materials at extreme temperatures.

Wear protection gloves and clothing.

Never eat, drink, or smoke in the laboratory.

Wash your hands before and after working.

Safety and Electromagnetic Capability (EMC) Standards

U.S. and Canadian Safety Standards	This instrument has been tested to and complies with standard UL 3101-1, "Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements."
	This instrument has been tested to and complies with standard CSA 1010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."
Canada EMC Standard	This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators
European Safety and EMC Standards 	<p>Safety</p> <p>This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements," and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."</p> <p>EMC</p> <p>This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."</p>
Australian EMC Standards 	This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Instrument Installation

Review the Environmental Parameters, Instrument Specifications and Safety Precautions in this User Guide before installing the BAX® System X5 instrument.

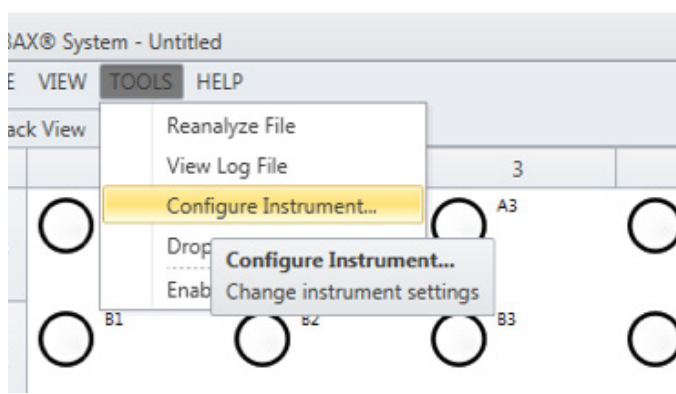
1. Review the laboratory space intended for the BAX® System X5 instrument and confirm that the appropriate space requirements are met (see “Environmental Parameters” and “Instrument Specifications” for details).
2. Open the flight case. Lift the BAX® System X5 instrument out of the flight case and place it on a solid, level surface.
3. Lift the bottom panel divider out of the flight case. Check to ensure that all components are present and intact.
4. Connect one end of the Ethernet cable to the Ethernet port of the BAX® System X5 computer. Connect the other end of the Ethernet cable to the Ethernet port on the rear of the BAX® System X5 instrument.

Note: A LAN network connection may also be used to connect the BAX® System X5 instrument and computer. To connect using an existing LAN, use the appropriate IP addresses provided by your network. The connections can be on any network ports in the same subnet and still allow the software to find the instrument. If necessary, hubs or switches can be used to create more access points.

5. Connect the AC adapter to the rear of the BAX® System X5 instrument.
6. Keep the flight case and packaging in case of return.

Instrument Configuration

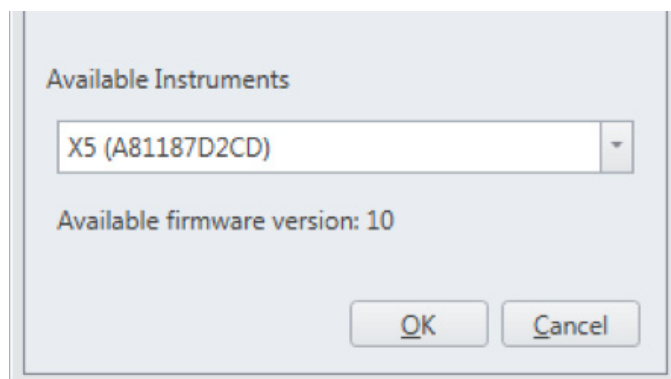
After installing a new BAX® System X5 instrument, you may need to configure the instrument for your software. You may also need to configure the instrument with your software if you are using multiple BAX® System X5 instruments with a single computer and switch between instruments.



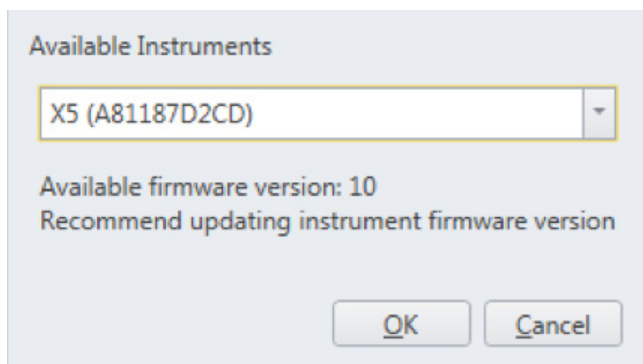
Ensure that the BAX® System X5 computer is connected to the BAX® System X5 instrument. When the instrument has a valid connection, you will see a green and yellow light above the connector.

From the BAX® System menu bar, select **TOOLS > CONFIGURE INSTRUMENT**.

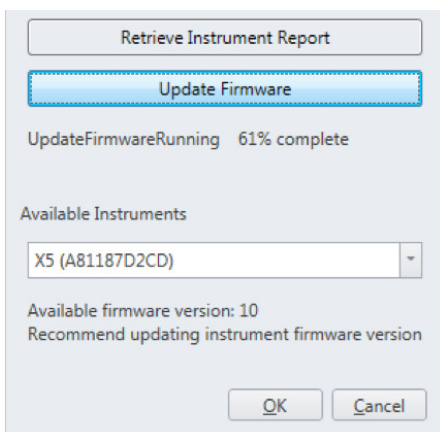
A dialog box opens to display information about the instrument.



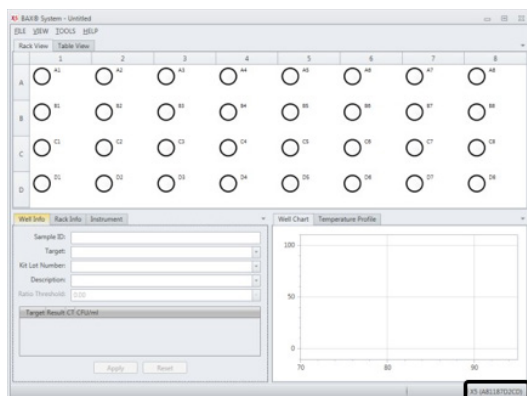
Under “Available Instruments”, use the drop-down menu to select the desired instrument. (In the example photo, instrument “A81187D2CD” is selected.) Click **OK** to confirm the selection.



Note: In some cases, the BAX[®] System X5 software may detect that the instrument does not have the latest firmware version. If a newer firmware version is available, the dialog box will display a message above the **OK** button that recommends updating instrument firmware version.



To update the instrument firmware version, click the **UPDATE FIRMWARE** button. A status appears in the dialog box to display the progress of the update. After the update is complete, click **OK** to confirm the instrument selection.



After configuration, the name of the selected BAX[®] System X5 instrument will appear in the bottom-right corner of the software screen.

Instrument Maintenance

The BAX® System X5 instrument does not require any regular maintenance procedures. If desired, basic cleaning and upkeep can be performed by following the instructions below.

Check the area around the BAX® System X5 instrument to ensure that the air flow is unrestricted.

Cleaning the Instrument Casing

Remove dust and debris by wiping the instrument surfaces with a lint-free cloth. If needed, the casing can be wiped off with a damp cloth using mild soap or common disinfectants, such as 70% ethanol. Do not use cleansers containing iodine or acetone.

Cleaning the Sample Wells

1. Use a lint-free swab soaked in 70% ethanol to gently clean the inside of the wells.
2. Dry the wells with a lint-free cloth or dry swab and remove small particles with an air duster.

Instrument Decontamination

When samples undergo PCR, the amplification process produces billions of copies of a specific DNA sequence from the targeted bacteria. If tubes are then unsealed, these copies can travel throughout the lab via aerosols, liquids and surface contact.

The BAX® System avoids this problem by processing only sealed tubes. However, as with any PCR technology, the risk of contamination cannot be completely eliminated. If the tubes are not properly sealed or are inadvertently opened after amplification, contamination of the lab should be assumed and care should be taken to minimize aerosols.

Follow these procedures to remove known amplicon contamination or as instructed by Diagnostics Support.

Decontaminating Sample Wells

1. Dilute a commercially available 5% bleach solution with pure water to create a 1% sodium hypochlorite solution.
2. Use a lint-free swab moistened with 1% sodium hypochlorite solution to thoroughly wet each sample well.
3. Let the solution sit in the sample wells for 10 minutes.
4. Use a lint-free swab moistened in pure water to wipe each sample well. Be sure to remove the sodium hypochlorite solution completely.
5. Wipe each sample well with a lint-free swab soaked in 70% ethanol.
6. Dry the wells and remove small particles with an air duster.

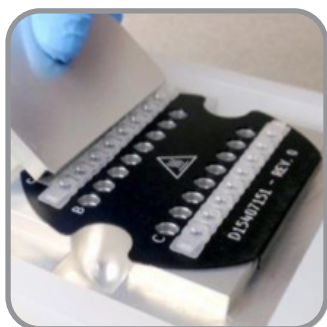
Decontaminating the Instrument Mount and Exterior Surfaces

1. Dilute a commercially available 5% bleach solution with pure water to create a 1% sodium hypochlorite solution.
2. Wipe the exterior surfaces of the BAX® System X5 instrument and the mount with a cloth dampened – but not dripping wet – with 1% sodium hypochlorite solution.
3. Let the solution sit on the instrument for 10 minutes.
4. Use a lint-free cloth moistened in pure water to thoroughly wipe the BAX® System X5 instrument and the mount. Be sure to remove the sodium hypochlorite solution completely.
5. Use a lint-free cloth soaked in 70% ethanol to wipe the BAX® System X5 instrument and the mount.
6. Let the instrument air-dry for a minimum of 10 minutes before using the instrument.

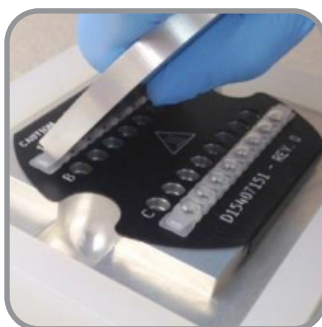
Capping/Decapping Tools

With your purchase of the BAX® System X5 start-up package, you receive a tool* that facilitates the decapping and resealing of the PCR tubes. This tool allows you to easily remove the domed caps from a strip of up to eight PCR tubes at once without jarring the contents of the tubes (A). The rounded end is used to reseal the tubes with flat optical caps using a rolling motion (B).

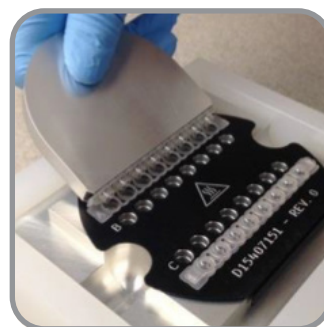
A. Removing domed caps from PCR tubes



Place tool flange behind row of caps

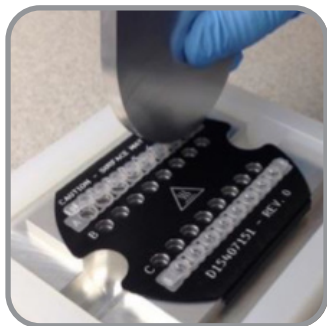


Tilt the tool forward and push down so that it locks over the caps.

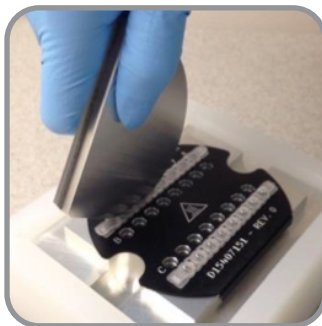


Tilt the tool backward so that the caps lift off.

B. Sealing PCR tubes with flat optical caps



Place rounded edge of tool over first cap.



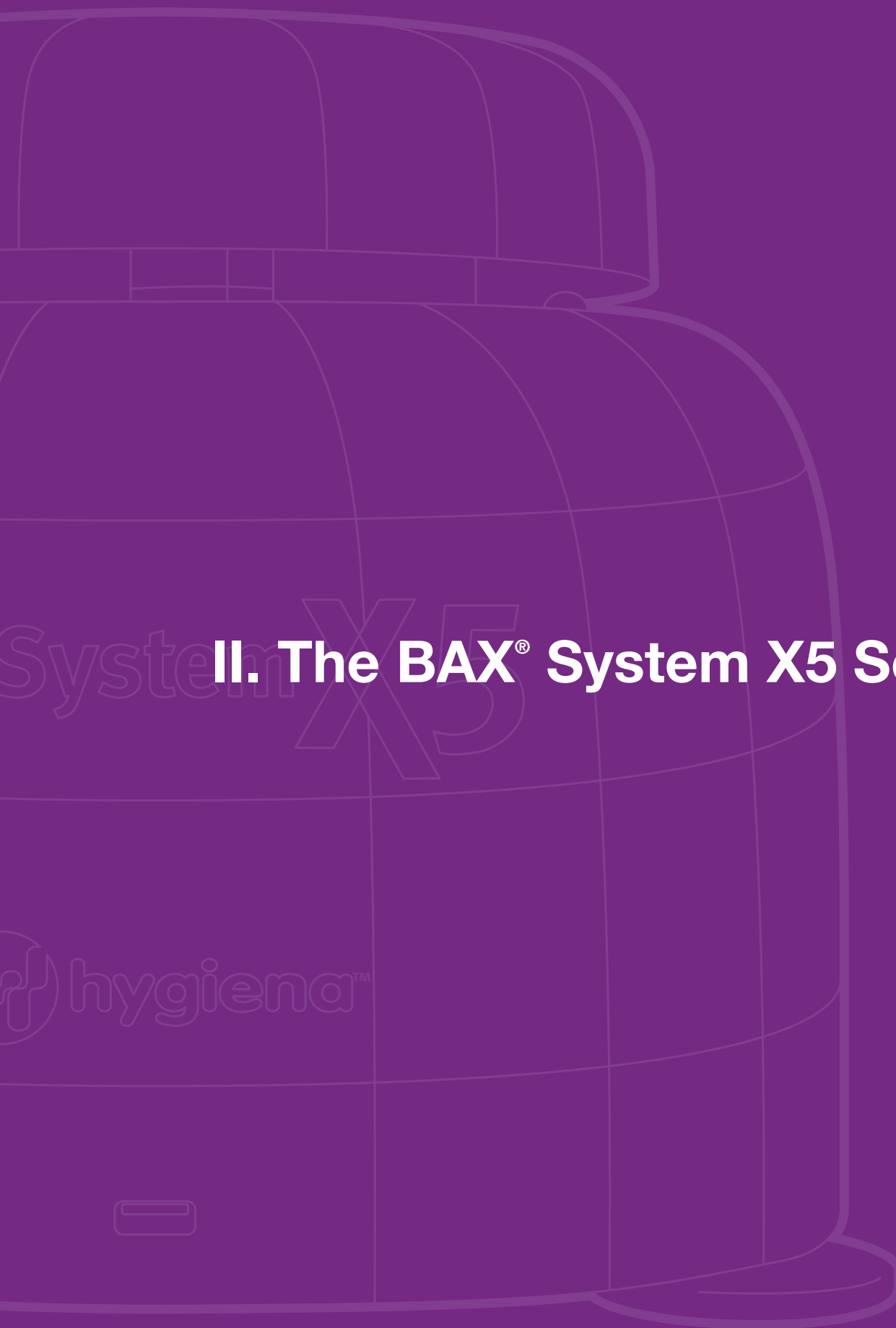
Roll the tool over the strip to lock the caps in place.



Visually inspect the caps to make sure they are seated.

*U.S. patent 5.967.001

Note: A separate capping/decapping tool is provided for use with cluster tubes for sample lysis.



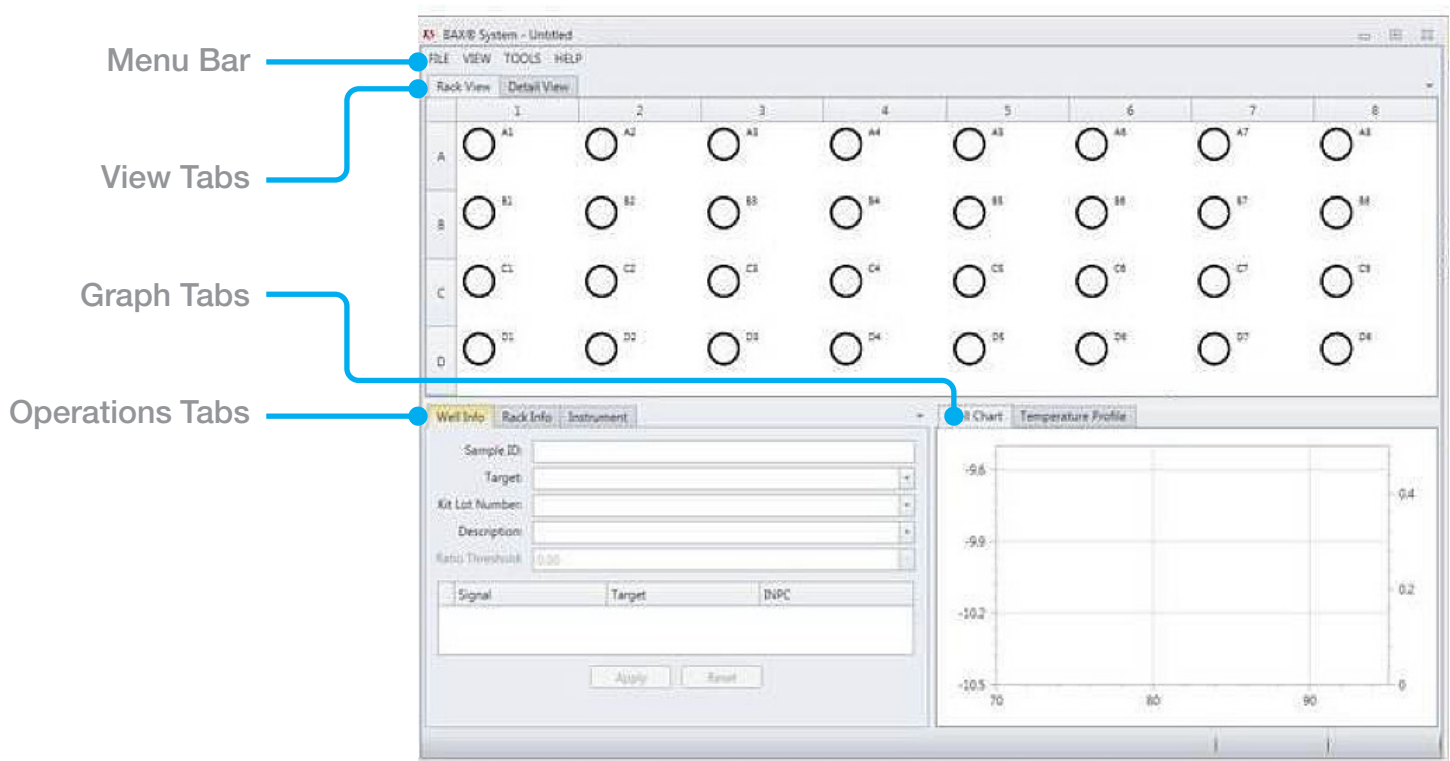
System X5

II. The BAX® System X5 Software

hygiena™

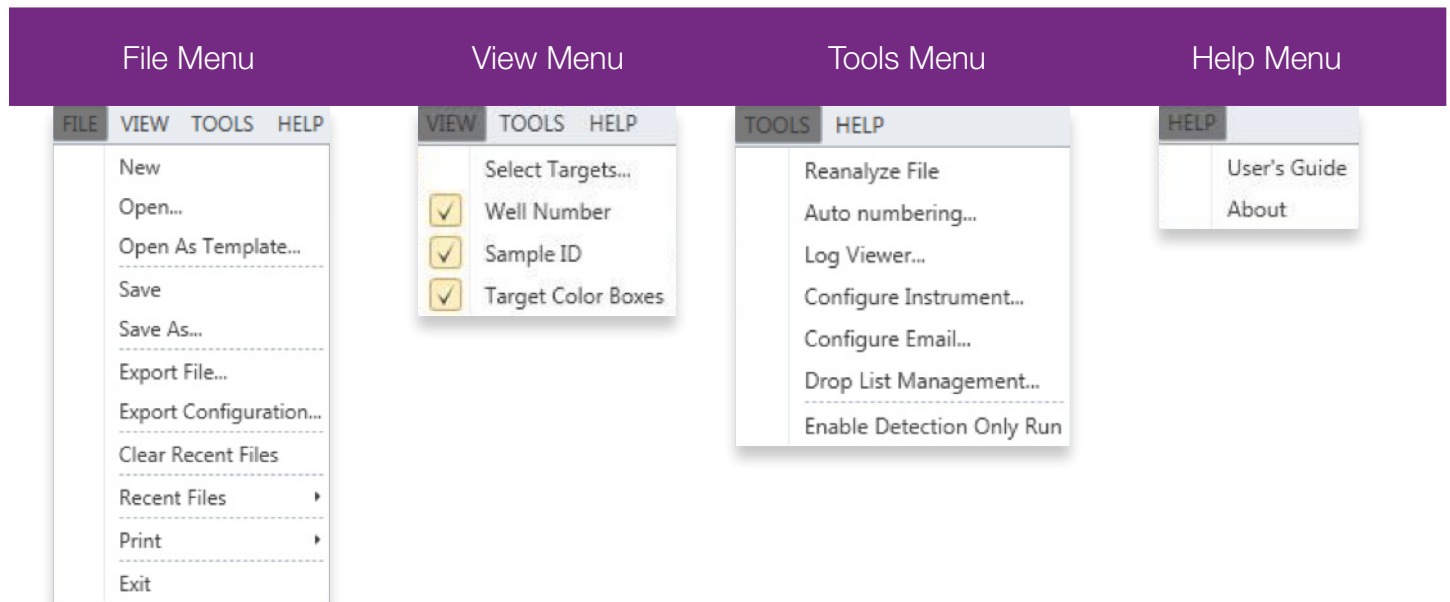
The BAX® System X5 Software Window

The main window is divided into these sections:



Menu Bar

The **Menu Bar** provides drop-down options for performing software functions and otherwise manipulating the file.



View Tabs

The **View Tabs** switch between Rack View and Table View of the sample setup and results.

	1	2	3	4	5	6
A	<input type="radio"/> A1	<input type="radio"/> A2	<input type="radio"/> A3	<input type="radio"/> A4	<input type="radio"/> A5	<input type="radio"/> A6
B	<input type="radio"/> B1	<input type="radio"/> B2	<input type="radio"/> B3	<input type="radio"/> B4	<input type="radio"/> B5	<input type="radio"/> B6

Rack View Tab

Well	Sample ID	Target	Result	Lot Number	Description
A1	<input type="radio"/>	Empty			
A2	<input type="radio"/>	Empty			
A3	<input type="radio"/>	Empty			

Detail View Tab

Operations Tabs

The **Operation Tabs** switch between viewing Well Info for a specific sample, Rack Info for the entire process run and Instrument status.

Well Info | Rack Info | Instrument

Sample ID:

Target:

Kit Lot Number:

Description:

Ratio Threshold:

Signal	Target	INPC

Apply Reset

The Well Info Tab allows you to enter information about each individual sample

Well Info | Rack Info | Instrument

Rack Status:

Accession:

Label:

Operator ID:

Description:

Created Time:

PCR Time:

Detection Time:

Analysis Time:

Verification Time:

Apply Reset

The Rack Info Tab allows you to enter information about all samples in the process run

Well Info | Rack Info | Instrument

Block temperature:

Phase:

Cycle:

Stage:

Time remaining:

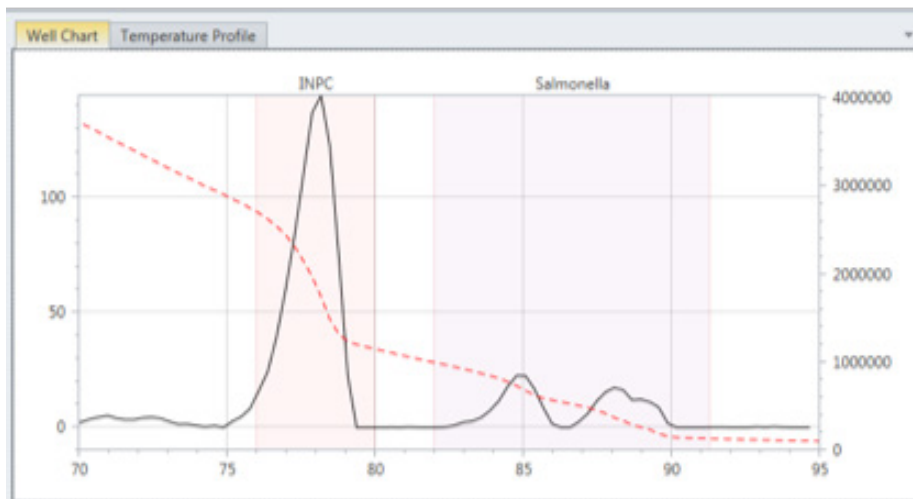
Run Cancel

Do not load samples until prompted.

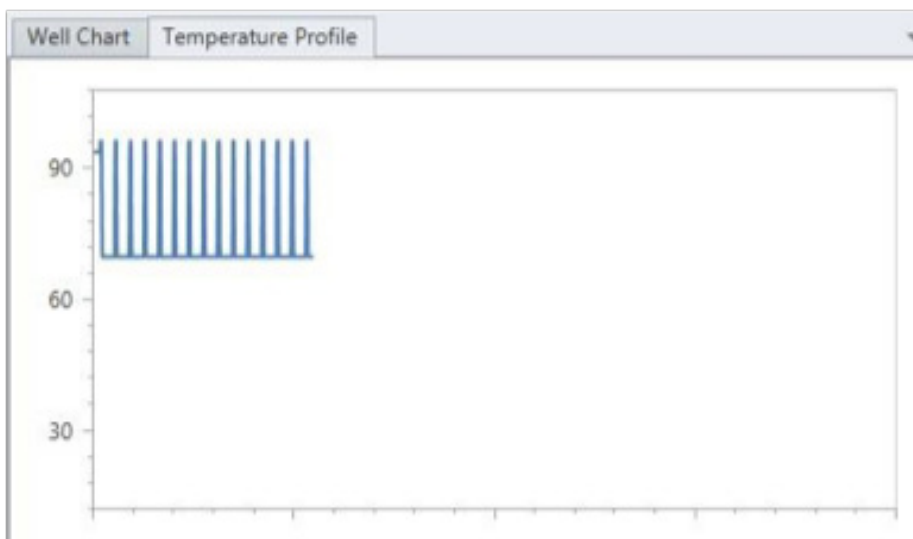
The Instrument Tab shows the current status of the BAX® System X5 instrument

Graph Tabs

The **Graph Tabs** switch between viewing the **Well Chart** (graph of the results for a specific sample) or the **Temperature Profile** (graph of the heating/cooling cycles during a process run)



After the process run is complete, the Well Chart displays a graphical representation of the sample results. The shaded regions denote different areas of interest in the assay.



During a process run, the Temperature Profile tab displays a live graph of the heating/cooling cycles. When the process run is complete, this tab is blank.

Creating a Rack File

Well Info | Rack Info | Instrument

Sample ID: 82246

Target: Genus Listeria

Kit Lot Number: Q4022

Description: Bench swab

Ratio Threshold: 0.00

Signal	Target	INPC

Apply Reset

In the Well Info Tab – Highlight the desired sample well (or wells) and enter information about each individual sample –

- **Sample ID** (optional) – Enter identifying information for the sample well
- **Target** (required) – Select the proper target for the sample from the drop-down menu
- **Kit Lot Number** (recommended) – Enter the lot number of the BAX® System assay you are using to process the samples
- **Description** (optional) – Enter a short description of the sample

Click **APPLY** to save the entered information.

Well Info | Rack Info | Instrument

Rack Status:

Accession:

Label: Zone 2

Operator ID: M. Smith

Description: Environmental Test - July 2015

Created Time:

PCR Time:

Detection Time:

Analysis Time:

Verification Time:

Apply Reset

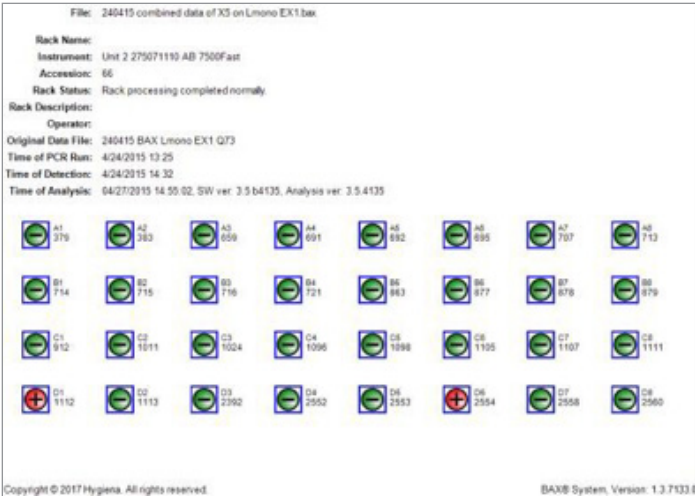
In the Rack Info Tab – Highlight the desired sample well (or wells) and enter information about all samples in the process run –

- **Label** (optional) – Enter identifying information for the entire sample rack
- **Operator ID** (optional) – Enter identifying information for the person setting up the process run
- **Description** (optional) – Enter a short description of the entire sample rack

Click **APPLY** to save the entered information.

Printing Reports

To print the results from a BAX® System X5 process run, select FILE > PRINT from the software Menu Bar. Results can be printed in five standard formats:



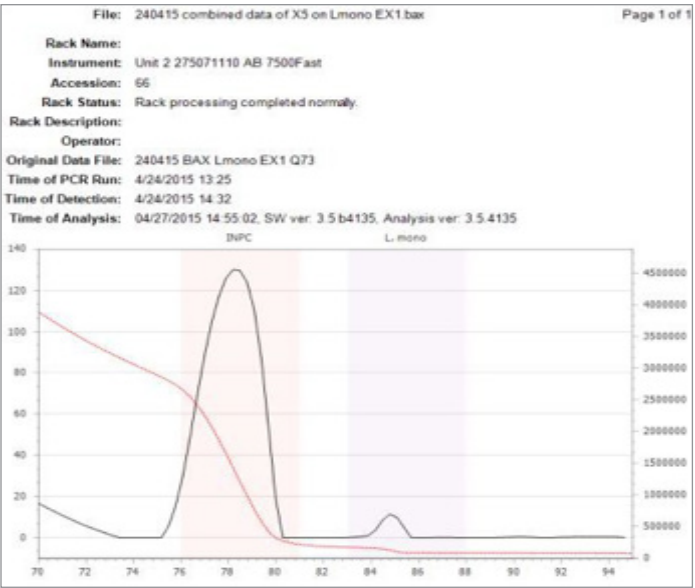
Rack Report – Prints a one-page summary of all samples in a process run, including the yes-or-no well results icons for each sample

File: 240415 combined data of X5 on Lmono EX1.bax

Rack Name:
Instrument: Unit 2 275071110 AB 7500Fast
Accession: 66
Rack Status: Rack processing completed normally.
Rack Description:
Operator:
Original Data File: 240415 BAX Lmono EX1 Q73
Time of PCR Run: 4/24/2015 13:25
Time of Detection: 4/24/2015 14:32
Time of Analysis: 04/27/2015 14:55:02, SW ver: 3.5 b4135, Analysis ver: 3.5.4135

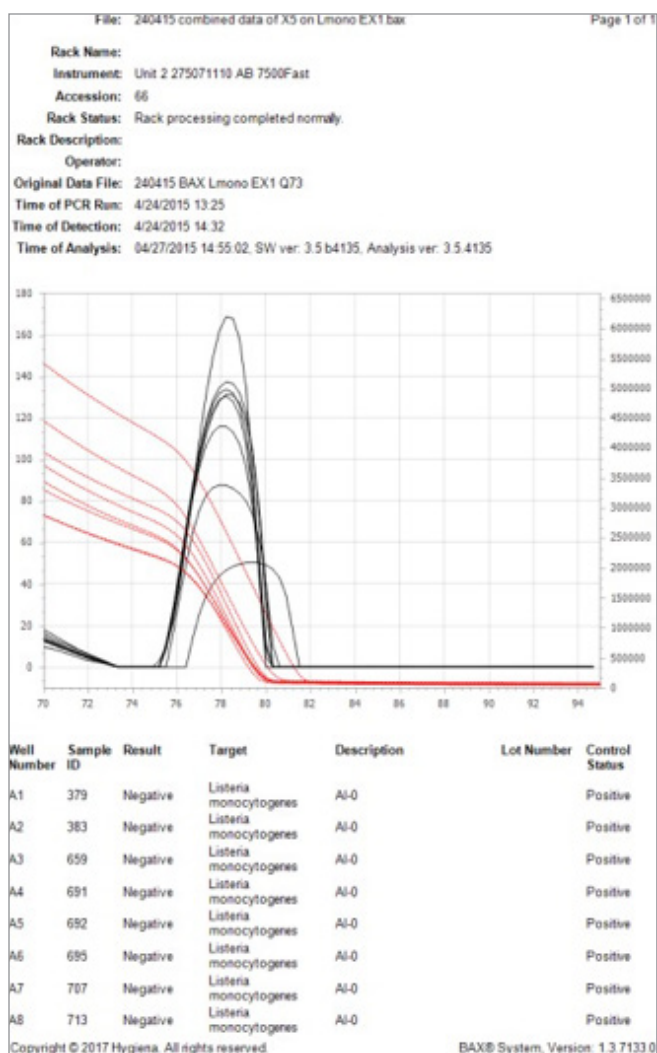
Well Number	Sample ID	Result	Target	Description	Lot Number	Control Status
D1	1112	Positive	Listeria monocytogenes	AI-0		Positive
D2	1113	Negative	Listeria monocytogenes	AI-0		Positive
D3	2392	Negative	Listeria monocytogenes	AI-0		Positive
D4	2552	Negative	Listeria monocytogenes	AI-0		Positive

Detailed Report – Prints a report that includes all information entered for each sample in the process run

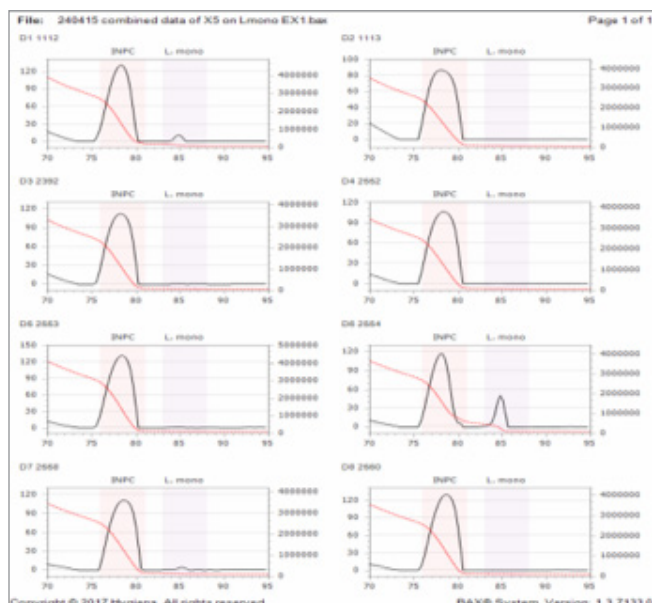


Individual Well Report – Prints a one-page report for an individual sample within a process run, including the graphical representation of the sample results from the Well Chart.

Note: To print individual well reports for multiple samples in a process run, you can highlight the desired wells in the View Tab before printing.



Overlay Well Report – Prints a one-page report for multiple wells, overlaying the graphs for comparison.

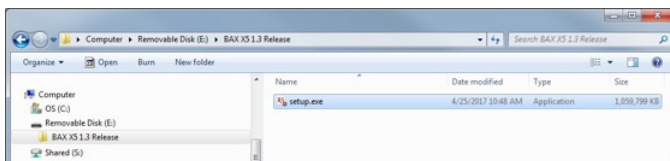


Graphical Report – Prints all selected wells in small individual graphs for comparison.

Installing and Upgrading the BAX® System Software

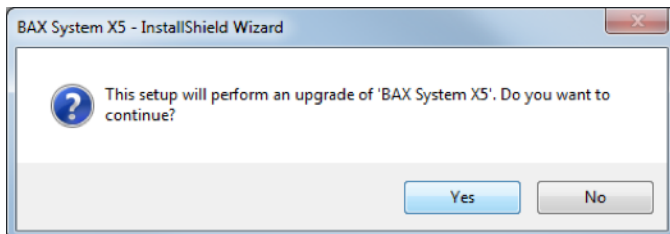
Because each release of BAX® System software builds on improvements from previous versions, it is strongly recommended that you always install the most recent version as soon as possible for optimal performance.

Note: The BAX® System software upgrade must be transferred to the BAX® System X5 computer, using a USB flash drive (memory stick), and/or network, or similar. The example below shows the process using a USB drive.



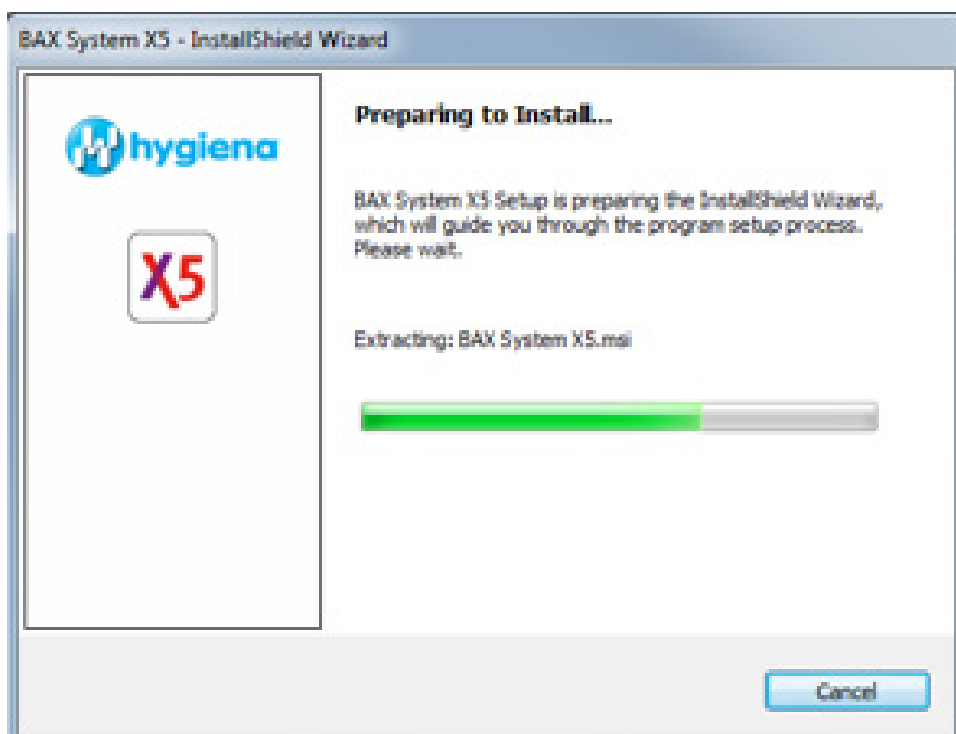
Insert a USB memory stick in the BAX® System X5 computer. Navigate to the folder that contains the BAX® System software installation files.

Double-click the file titled “BAX Setup.exe” to begin installation.

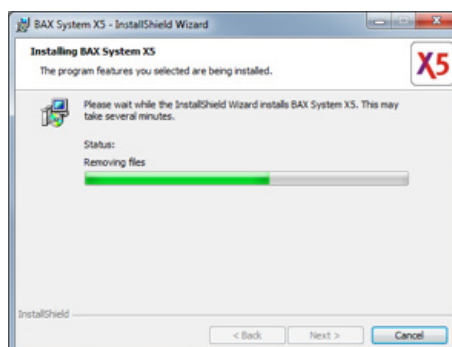
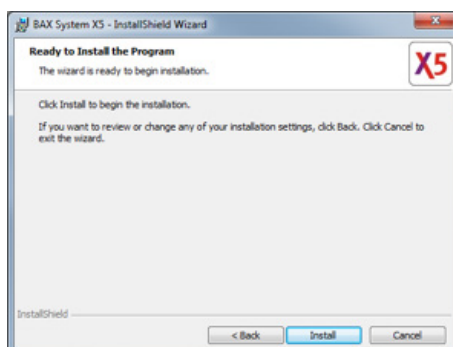


If you are doing an upgrade, a confirmation window may appear asking if you want to continue with the upgrade. Click **YES** to begin the upgrade installation.

Note: You may also be asked some security questions by Windows, depending on the computer's settings and your account privileges. Allow the installation to modify the computer if so.

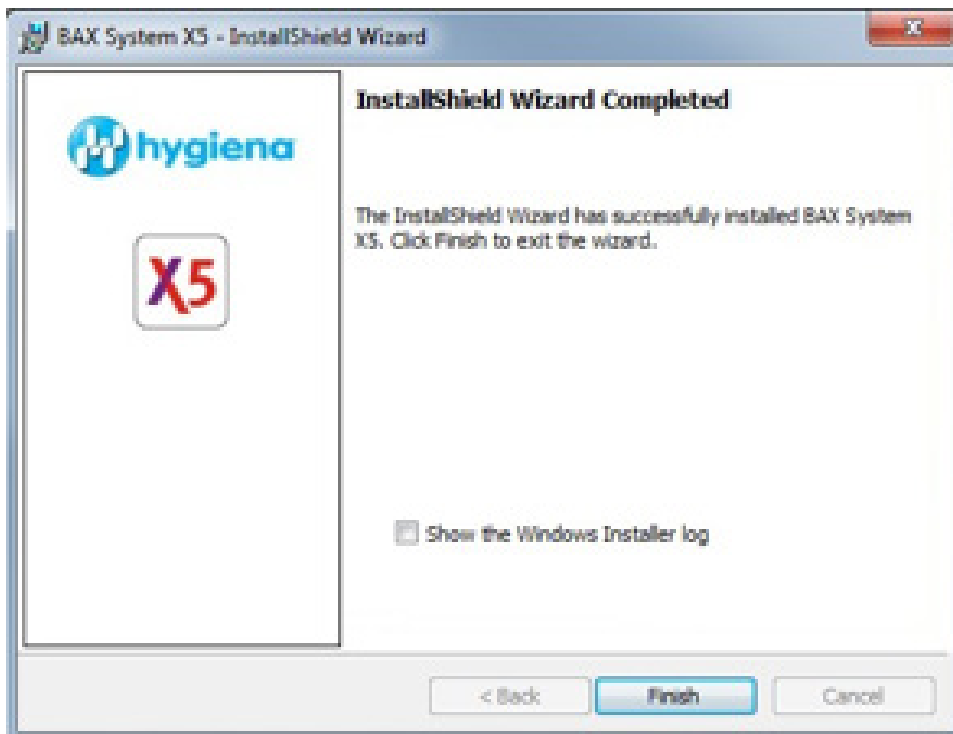


The installation wizard begins and proceeds through a number of installation screens – some examples are shown here.

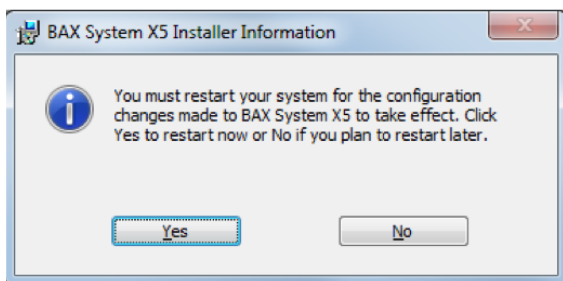


There may be different screens depending upon whether this is a new install or an upgrade. Click **OK** or **NEXT** to accept any modifications required for the software installation.

II. The BAX® System X5 Software



After the installation wizard is complete, click **FINISH** to end the installation.



Re-start the BAX® System X5 computer to complete the software install and apply all changes.

Click **YES** to re-start the computer immediately after the software install is complete, or click **NO** to re-start the computer at a later time.

III. The BAX® System X5 PCR Assays

hygiena™

III. The BAX® System X5 PCR Assays

Each BAX® System PCR assay includes everything required for preparing sample enrichments, including lysis buffer and protease for sample preparation and strips of PCR tubes containing BAX® System PCR tablets for automated processing in the BAX® System instrument. These PCR tablets combine all the requisite reaction reagents, including fluorescent dye and an internal positive control, into a stable, dry tablet for simplicity and convenience - simply hydrate them with prepared sample and load the rack into the BAX® System instrument.

Assay Validation

The protocols in this chapter have been validated by Hygiena for use with the BAX® System assays. In order to follow the BAX® System method, the enrichment and sample preparation protocols must be followed exactly as described in this user guide and the kit instructions.

For many sample types, standard enrichment protocols (such as the USDA-FSIS, FDA-BAM or ISO methods) may also be used. These enrichment protocols should be internally validated before use with the BAX® System, as these methods have not been validated by Hygiena or any third-party organizations. To test a sample type that is not listed, please contact Hygiena Diagnostics Support for recommendations on enrichment and sample preparation.

Using the *E. coli* O157:H7 Assay

1. Enrich Samples

Assay Kit #KIT2022



The **AOAC Research Institute** has certified the BAX® System for detecting *E. coli* O157:H7 in raw ground beef, beef trim, spinach and lettuce.

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Enrichment	Secondary Enrichment
Raw ground beef	<p>For 25 g – Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.</p> <p>For 65 g – Homogenize 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.</p>	None
Beef trim	<p>For 65 g – Gently massage 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media for 30 seconds so that broth covers entire surface of sample. Incubate at 42°C for 8-24 hours.</p> <p>For 325 g – Gently massage 325 g sample with 975 mL pre-warmed (18-35°C) mTSB media for 30 seconds so that broth covers entire surface of sample. Incubate at 42°C for 20-24 hours.</p> <p>For 375 g – Gently massage 375 g sample with 1.5 L pre-warmed (45°C) BAX® System MP media for 30 seconds so that broth covers entire surface of sample. Incubate at 42°C for 10-24 hours.</p>	None
Spinach and lettuce	Combine 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media and swirl to soak entire sample. Incubate at 42°C for 8-24 hours.	None
Red leaf lettuce	<p>For 200 g – Combine 200 g sample with 450 mL of mBPWp media and swirl to soak entire sample. Incubate at 37°C for 5 hours. After incubation 2 mL of ACV supplements and incubate at 42°C for an additional 18-24 hours.</p> <p>For 375 g – Combine 375 g sample with 1.5 L pre-warmed (42°C) BAX® System MP media and swirl to soak entire sample. Incubate at 42°C for 10-22 hours.</p>	<p>For 200 g Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.</p> <p>375 g – None</p>

1. Enrich Samples (Continued)



The **French Association of Normalization (AFNOR Certification)** has certified the BAX[®] System PCR Assay for *E. coli* O157:H7 MP according to the NF VALIDATION rules for detecting *E. coli* O157:H7 in raw beef, raw pork, ovine and chicken meats, raw milk, fruits and vegetables, ready to eat and ready to reheat dishes.

Sample type	Enrichment
Raw beef meats	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX [®] System MP media. Incubate at 42°C for 8-24 hours.
Raw milk, fruits and vegetables, ready to eat and ready to reheat dishes, raw pork, ovine and chicken meat	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) mTSB broth supplemented with novobiocin (20 mg/L). Incubate at 41.5°C for 18-24 hours.

2. Prepare BAX[®] System Lysis Reagent

Note: Do not over-mix the lysis reagent, as this can cause the mixture to foam.

To create a full bottle of BAX[®] System lysis reagent (prepares about 60 samples)

1. Add 150 µL of protease to one 12 mL bottle of lysis buffer.
2. Cap the bottle and invert it several times to mix.

To create smaller volumes of BAX[®] System lysis reagent (prepares about 4 samples)

1. Add 12.5 µL protease to 1 mL of lysis buffer in a separate sterile container.
2. Seal the container and invert it several times to mix.

3. Perform Sample Lysis

1. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
2. Create a rack file according to the instructions found under “Creating a Rack File”.
3. Turn on the Automated Thermal Block and select the Gram Negative program.

Note: Lysis may also be performed using separate analog heating and cooling blocks. See “Using Analog Heating and Cooling Blocks with the BAX[®] System Assays” for details and protocols.

4. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
5. Transfer 200 µL lysis reagent to each of the cluster tubes.

Note: Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.

6. For all other samples, transfer 5 µL enriched sample.
 - a. Transfer enriched sample to the corresponding cluster tubes, using a new pipette tip for each sample.
 - b. For samples enriched in BAX[®] System MP media, transfer 20 µL enriched sample.
7. After all transfers have been completed, secure the caps.
8. After the “Load Samples” prompt appears on the Automated Thermal Block, place the rack of cluster tubes onto the Automated Thermal Block.
9. Press the **SELECT/CONTINUE** button to begin automated lysis.

Note: This process takes about 1 hour to complete.

10. When sample lysis is complete, the Automated Thermal Block displays the message “Sample PCR Ready” and sounds four audible beeps.

4. Hydrate PCR Tablets

1. Select a chilled (2-8°C) PCR cooling block.

Note: Remember that you must finish using the cooling block within 30 minutes.

Note: Users following Health Canada's MFLP-30, use PCR cooling block which have been frozen at -20°C.

2. Place a 32-well PCR tube rack over the insert.
3. Arrange strips of PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
4. Remove the aluminum block with the cluster tubes of sample lysate from the Automated Thermal Block and place it next to the PCR cooling block. Press the **SELECT/CONTINUE** button on the Automated Thermal Block to complete the program.
5. Remove the caps from the first strip of PCR tubes with the decapping tool.
6. Transfer 50 µL lysate into PCR tubes, then seal with flat optical caps.
7. Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated and sealed.

5. Complete a BAX® System Process Run

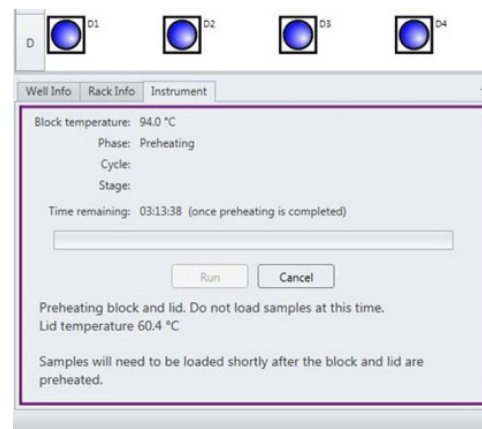
Take the rack of PCR tubes in the cooling block to the BAX® System X5 instrument.

In the BAX® System X5 software window, select the Info Tab labeled “Instrument” to view the current instrument status.

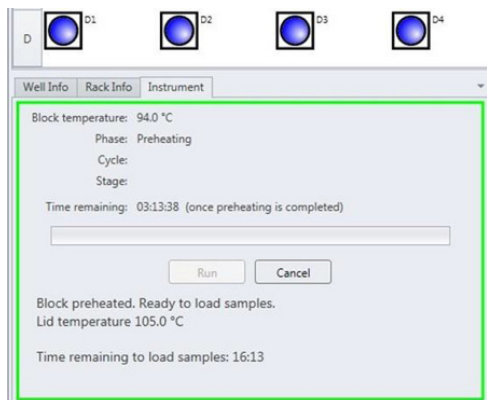
Click **RUN** to begin pre-heating the instrument.

Note: If you have not created and saved a rack file, you will be prompted to do so before the instrument begins pre-heating.

Note: Samples must be loaded within 20 minutes after the RUN button is clicked. If samples are not loaded in 20 minutes, the process run times out and you will need to re-start the process.



When the instrument is heated, the instrument lid LED will turn green and the instrument prompt under the status bar reads “Block preheated. Ready to load samples.”



Remove the tube rack with PCR tubes from the cooling block. Visually check PCR tubes to ensure the liquid contains no air bubbles and tubes are clean.

- a. **IMPORTANT:** If air bubbles are present in the liquid, gently tap or flick the tubes to allow all air bubbles to escape.
- b. If the outside of the PCR tubes have dust, oils or residue, clean the outside of each PCR tube with a clean lab wipe.

Open the BAX® System X5 instrument lid and load the PCR tubes into the instrument mount according to the setup of your rack file.

III. The BAX® System X5 PCR Assays

E. coli O157:H7 Assay

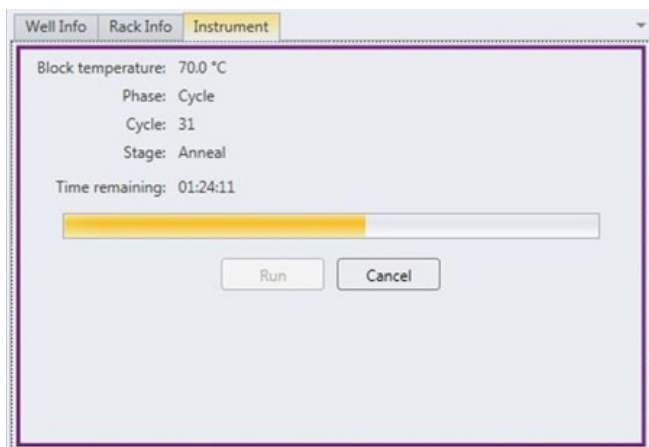
5. Complete a BAX® System Process Run (Continued)

To process fewer than 32 samples, load samples first in Row A and Row D, starting at the corners.



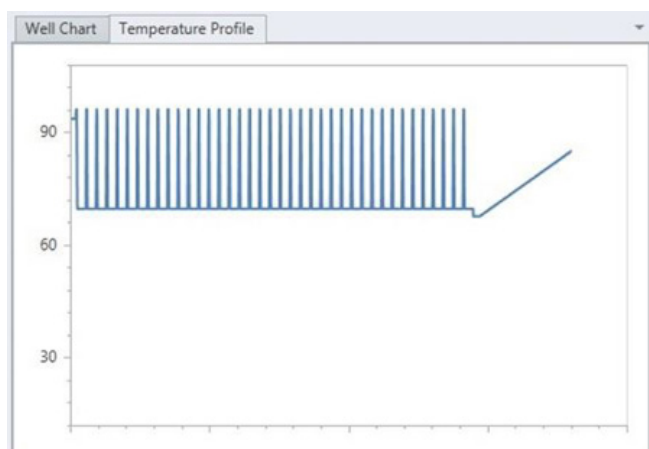
Once the two outer rows are full, then use Row B and Row C to load samples in a symmetric pattern to ensure the instrument mount remains balanced.

Close the instrument lid as soon as possible to prevent the instrument from cooling. The process run begins automatically.



Note: After the instrument lid is closed, it may take a few moments for the instrument to stabilize before the run begins. When the instrument is stabilized, the process run begins automatically.

As the run progresses, the Instrument tab displays a status bar and estimates the time remaining before results can be viewed.



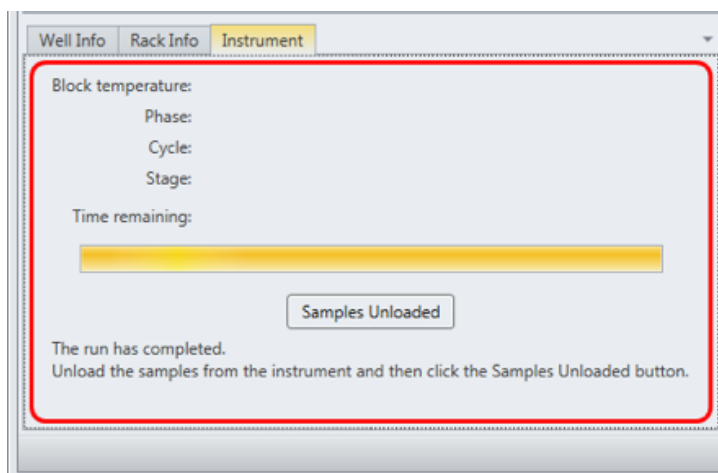
During the process, the instrument temperature is displayed graphically in the Graph Tab labeled “Temperature Profile”.

Note: Variations in the temperature profile between different process runs are normal and do not affect results.

5. Complete a BAX[®] System Process Run (Continued)

After processing is complete, the instrument prompt under the status bar reads “The run has completed”.

Open the instrument lid and remove the PCR tubes from the instrument mount. Close the instrument lid.



Click the **SAMPLES UNLOADED** button to view results. The temperature profile disappears after run is complete.

6. Review Results

Note: For more information about reading BAX[®] System results, see “Reviewing BAX[®] System Results”.

When processing in the BAX[®] System X5 instrument is complete, a new window appears showing each color-coded well with an icon in the center to illustrate the result:

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

III. The BAX® System X5 PCR Assays

E. coli O157:H7 Assay

Positive *E. coli* O157:H7 MP Melting Curve Profiles

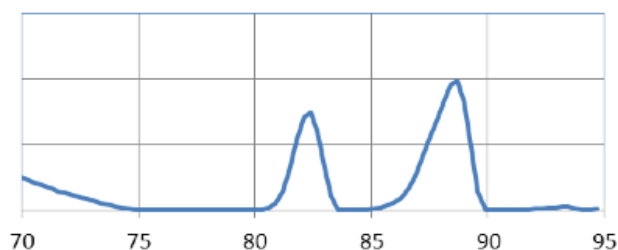
Samples that are positive for *E. coli* O157:H7 will display two target peaks.

These target peaks usually appear between 81-90°C.

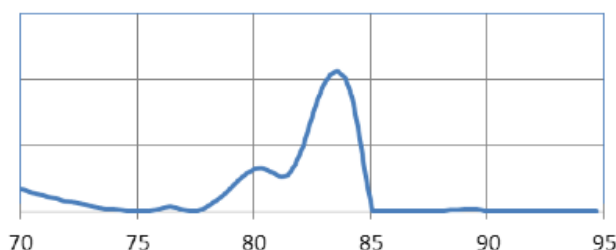
The graph also displays one INPC, or control peak, which usually appears between 79-82°C.

If a sample displays a strong positive result, the INPC may be very small in comparison to the target peaks.

Examples of Typical Melt Curves for Positive *E. coli* O157:H7 Samples



Strong X5 *E. coli* O157:H7 positive



Weak X5 *E. coli* O157:H7 positive

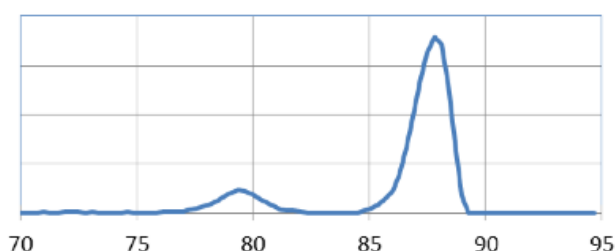
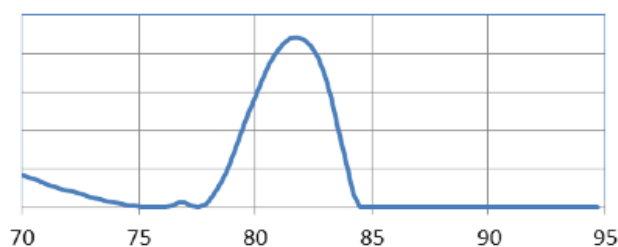
Negative *E. coli* O157:H7 MP Melting Curve Profiles

Samples that are negative for *E. coli* O157:H7 will display either one target peak on the melt curve (usually between 81-90°C), or no target peaks.

The graph also displays one INPC, or control peak, which usually appears between 79-82°C.

If a large target peak is also present, the INPC may be very small in comparison to the target peak.

Examples of Typical Melt Curves for Negative *E. coli* O157:H7 Samples



7. Confirm Positive Results

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX[®] System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX[®] System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Using the Genus *Listeria* Assay

1. Enrich Samples

Assay Kit #KIT2024



The **AOAC Research Institute** has certified the BAX® System for detecting Genus *Listeria* on environmental surfaces (stainless steel, plastic, ceramic tile, rubber, painted and unpainted wood, drain swabs, sealed concrete, cast iron, and air filter material) and on foods (dairy, meat, fish and vegetables).

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
BAX® System <i>Listeria</i> media		
Environmental sponges	Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with 10 mL D/E Neutralizing Broth or equivalent. Homogenize sponge with 190 mL pre-warmed (36°C) BAX® System <i>Listeria</i> media broth. Incubate at 36°C for 26-30 hours.	None
Environmental swabs	Sample a 1 x 1 in (2.5 x 2.5 cm) environmental area with a swab pre-moistened with 300-500 µL D/E Neutralizing Broth or equivalent. Add the swab to 10 mL pre-warmed (36°C) BAX® System <i>Listeria</i> media broth. Incubate at 36°C for 22-26 hours.	None
Standard Media		
Environmental sponges	Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with D/E Neutralizing Broth. Homogenize sponge with 190 mL pre-warmed (30°C) Demi-Fraser broth or UVM. 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.
Frankfurters	<p>For 25 g – Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.</p> <p>For 125 g – Homogenize 125 g samples with 1.125 L of pre-warmed (30°C) UVM media. Incubate at 30°C for 23-26 hours.</p>	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Standard Media		
Smoked salmon	Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser 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.
Spinach and other vegetables	<p>Demi-Fraser broth: Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.</p> <p>BLEB: Homogenize 25 g samples with 225 mL of pre-warmed (30°C) BLEB and incubate for 4 hours at 30°C. After 4 hours add selective agents acriflavine, nalidixic acid, and cycloheximide and incubate at 30°C for an additional 18-22 hours.</p>	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Cheese	Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser 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.
Queso fresco	Homogenize 25 g samples with 225 mL of pre-warmed (30°C) BLEB and incubate for 4 hours at 30°C. After 4 hours add selective agents acriflavine, nalidixic acid, and cycloheximide and incubate at 30°C for an additional 18-22 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.



QUA 18/03-11/02
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
<http://nf-validation.afnor.org>

The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting Genus *Listeria* in all human food products and environmental samples (excluding samples from primary production).

Sample type	Enrichment
Meat, dairy, vegetables, seafood (except smoked fish)	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
Smoked fish, raw & cooked delicatessen	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media with buffer supplement. Incubate at 37°C for 24-28 hours.
Environmental samples	Swab a 4 x 4 in (10 x 10 cm) area with a sponge pre-moistened in D/E neutralizing broth. Homogenize 1 sponge with 90 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.

2. Prepare BAX[®] System Lysis Reagent

Note: Do not over-mix the lysis reagent, as this can cause the mixture to foam.

To create a full bottle of BAX[®] System lysis reagent (prepares about 60 samples)

1. Add 150 μ L of protease to one 12 mL bottle of lysis buffer.
2. Add 200 μ L Lysing Agent 2 to the protease and lysis buffer mixture.
3. Cap the bottle and invert it several times to mix.

To create smaller volumes of BAX[®] System lysis reagent (prepares about 4 samples)

1. Add 12.5 μ L protease to 1 mL of lysis buffer in a separate sterile container.
2. Add 16.5 μ L Lysing Agent 2 to the protease and lysis buffer mixture.
3. Seal the container and invert it several times to mix.

3. Perform Sample Lysis

1. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
2. Create a rack file according to the instructions found under “Creating a Rack File”.
3. Turn on the Automated Thermal Block and select the RT Listeria program.

Note: Lysis may also be performed using separate analog heating and cooling blocks. See “Using Analog Heating and Cooling Blocks with the BAX® System Assays” for details and protocols.

4. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
5. Transfer 200 µL lysis reagent to each of the cluster tubes.
6. Transfer 5 µL enriched sample to the corresponding cluster tubes, using a new pipette tip for each sample.

Note: Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.

7. After all transfers have been completed, secure the caps.
8. After the “Load Samples” prompt appears on the Automated Thermal Block, place the rack of cluster tubes onto the Automated Thermal Block.
9. Press the **SELECT/CONTINUE** button to begin automated lysis.

Note: This process takes about 1 hour to complete.

10. When sample lysis is complete, the Automated Thermal Block displays the message “Sample PCR Ready” and sounds four audible beeps.

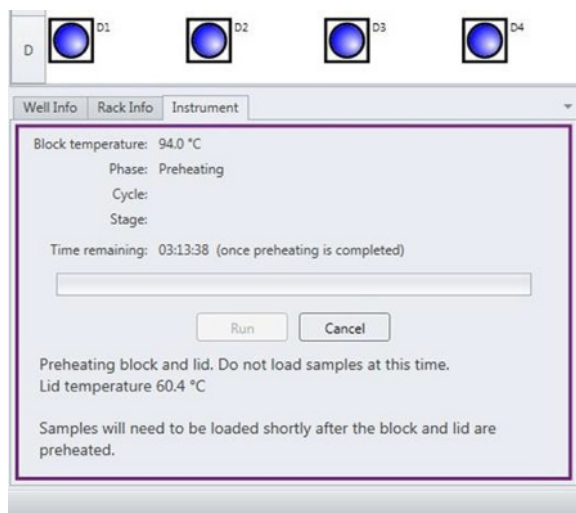
4. Hydrate PCR Tablets

1. Select a chilled (2-8°C) PCR cooling block.

Note: Remember that you must finish using the cooling block within 30 minutes.

2. Place a 32-well PCR tube rack over the insert.
3. Arrange strips of PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
4. Remove the aluminum block with the cluster tubes of sample lysate from the Automated Thermal Block and place it next to the PCR cooling block. Press the **SELECT/CONTINUE** button on the Automated Thermal Block to complete the program.
5. Remove the caps from the first strip of PCR tubes with the decapping tool.
6. Transfer 50 µL lysate into PCR tubes, then seal with flat optical caps.
7. Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated and sealed.

5. Complete a BAX® System Process Run



Take the rack of PCR tubes in the cooling block to the BAX® System X5 instrument.

In the BAX® System X5 software window, select the Info Tab labeled “Instrument” to view the current instrument status.

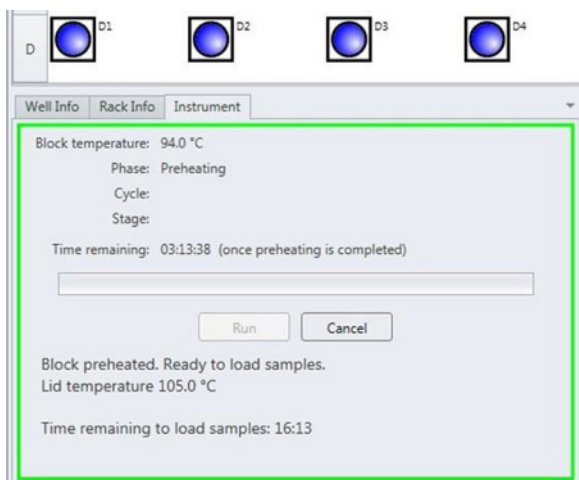
Click **RUN** to begin pre-heating the instrument.

Note: If you have not created and saved a rack file, you will be prompted to do so before the instrument begins pre-heating.

Note: Samples must be loaded within 20 minutes after the RUN button is clicked. If samples are not loaded in 20 minutes, the process run times out and you will need to re-start the process.

When the instrument is heated, the instrument lid LED will turn green and the instrument prompt under the status bar reads “Block preheated. Ready to load samples.”

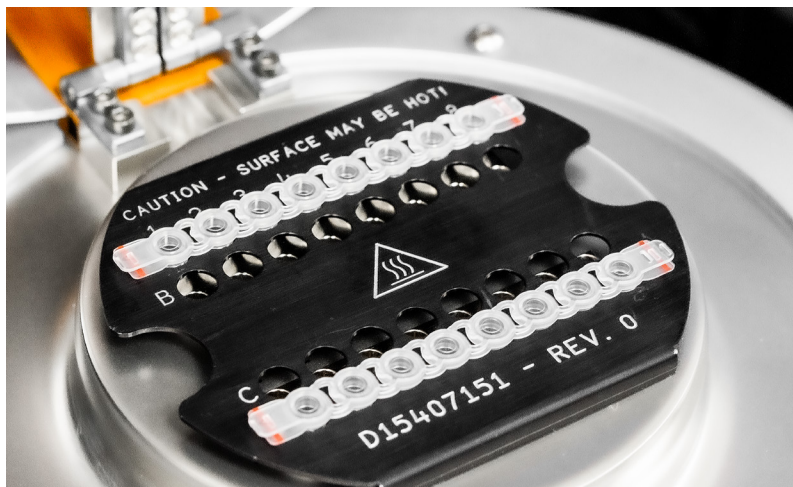
Remove the tube rack with PCR tubes from the cooling block. Visually check PCR tubes to ensure the liquid contains no air bubbles and tubes are clean.



- IMPORTANT:** If air bubbles are present in the liquid, gently tap or flick the tubes to allow all air bubbles to escape.
- If the outside of the PCR tubes have dust, oils or residue, clean the outside of each PCR tube with a clean lab wipe.

Open the BAX® System X5 instrument lid and load the PCR tubes into the instrument mount according to the setup of your rack file.

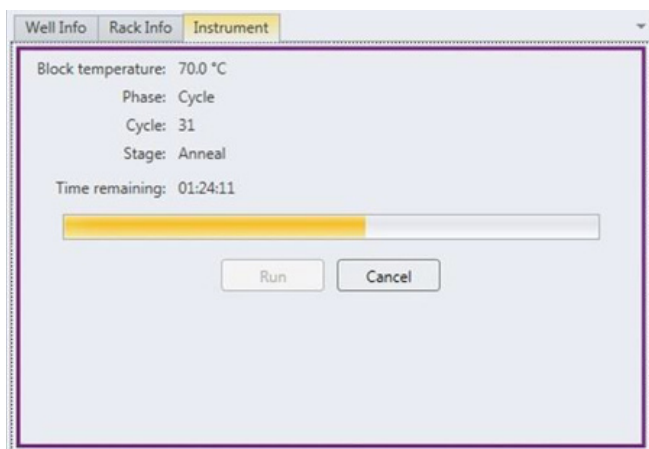
5. Complete a BAX® System Process Run (Continued)



To process fewer than 32 samples, load samples first in Row A and Row D, starting at the corners.

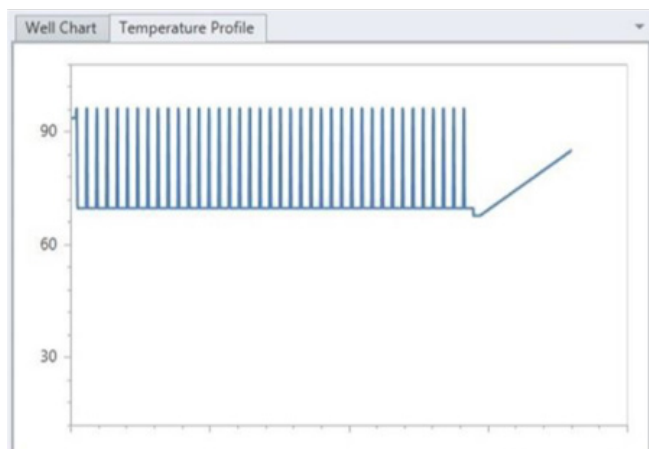
Once the two outer rows are full, then use Row B and Row C to load samples in a symmetric pattern to ensure the instrument mount remains balanced.

Close the instrument lid as soon as possible to prevent the instrument from cooling. The process run begins automatically.



Note: After the instrument lid is closed, it may take a few moments for the instrument to stabilize before the run begins. When the instrument is stabilized, the process run begins automatically.

As the run progresses, the Instrument tab displays a status bar and estimates the time remaining before results can be viewed.



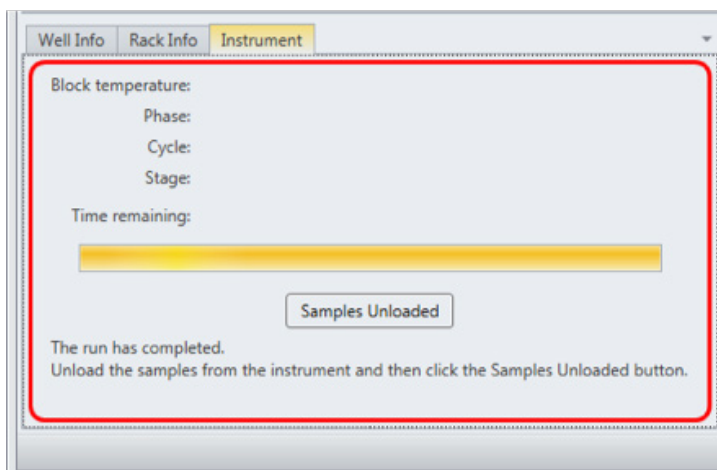
During the process, the instrument temperature is displayed graphically in the Graph Tab labeled "Temperature Profile".

Note: Variations in the temperature profile between different process runs are normal and do not affect results.

5. Complete a BAX® System Process Run (Continued)

After processing is complete, the instrument prompt under the status bar reads “The run has completed”.

Open the instrument lid and remove the PCR tubes from the instrument mount. Close the instrument lid.




Click the **SAMPLES UNLOADED** button to view results. The temperature profile disappears after run is complete.

6. Review Results

Note: For more information about reading BAX® System results, see “Reviewing BAX® System Results”.

When processing in the BAX® System X5 instrument is complete, a new window appears showing each color-coded well with an icon in the center to illustrate the result:

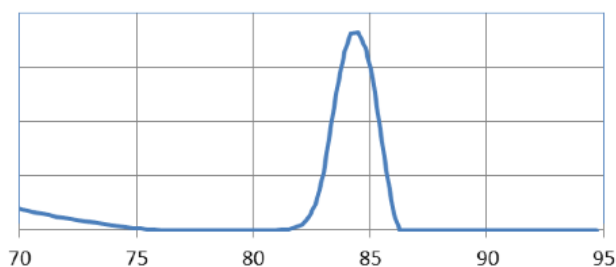
	Green (-)	=	Negative result
	Red (+)	=	Positive result
	Yellow (?)	=	Indeterminate result
	Yellow (?) with red slash	=	Signal error

Positive Genus *Listeria* Melting Curve Profiles

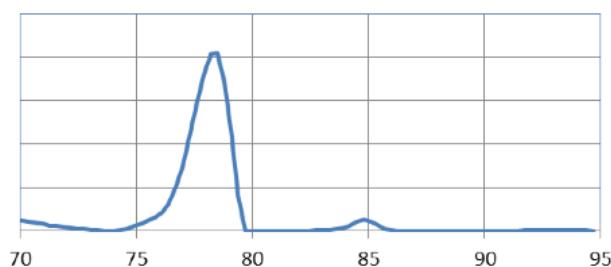
Samples that are positive for *Listeria* species will display one target peak. This peak usually appears between 83- 88°C. The target peak may appear as a “double peak” based on the *Listeria* strain present.

The graph also displays one INPC, or control peak, which usually appears between 76-81°C. If a sample displays a strong positive result, the INPC may be very small in comparison to the target peak.

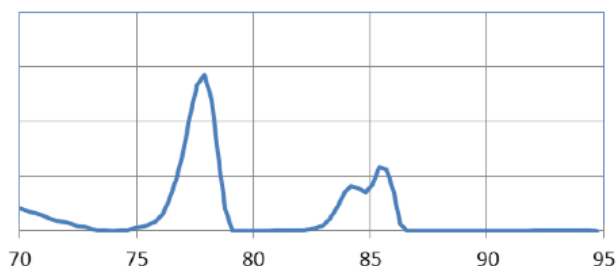
Examples of Typical Melt Curves for Positive Genus *Listeria* Samples



Strong X5 Genus *Listeria* positive



Weak X5 Genus *Listeria* positive

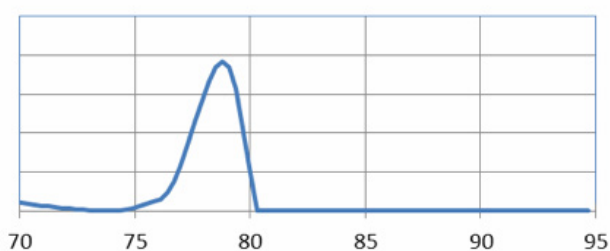


X5 Genus *Listeria* positive with double target peaks

Negative Genus *Listeria* Melting Curve Profile

Samples that are negative for *Listeria* species will display no target peaks. The graph displays only the INPC, or control peak, which usually appears between 76-81°C.

Example of Typical Melt Curves for Negative Genus *Listeria* Samples



7. Confirm Positive Results

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Using the *Listeria monocytogenes* Assay

1. Enrich Samples

Assay Kit #KIT2023



The **AOAC Research Institute** has certified the BAX® System for detecting *Listeria monocytogenes* in a wide variety of foods, including raw meats, fresh produce/vegetables, processed meats, seafood, dairy cultured/non-cultured, egg and egg products, fruit juices and plastic surfaces.

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
Ground beef, ground pork, ground chicken, deli turkey	<p>Demi-Fraser broth: Homogenize 25 g sample with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours.</p> <p>UVM broth: Homogenize 25 g sample with 225 mL pre-warmed (30°C) UVM broth. Incubate at 30°C for 20-24 hours.</p>	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Frankfurters	<p>For 25 g – Homogenize 25 g sample with 225 mL pre-warmed (30°C) BLEB without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours.</p> <p>For 125 g – Homogenize 125 g samples with 1.125L of pre-warmed (30°C) UVM media. Incubate at 30°C for 23- 26 hours.</p>	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Fish sticks, surimi, langostinos	Homogenize 25 g sample with 225 mL pre-warmed (30°C) BLEB without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 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	UPB: Homogenize 25 g samples with 225 mL pre-warmed Universal Pre-enrichment Broth. Incubate at 35°C or 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.
Ice cream, milk (2%) yogurt, soft cheese	Homogenize 25 g sample with 225 mL pre-warmed (30°C) Complete Selective Enrichment 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.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Cabbage slaw, peas, spinach, strawberries, apple juice and orange juice, queso fresco cheese, pepperoni	Homogenize 25 g sample with 225 mL pre-warmed (30°C) BLEB without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Environmental sponges	Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with D/E Neutralizing Broth. Homogenize sponge with 225 mL pre-warmed (30°C) UVM. Incubate at 30°C for 20-26 hours.	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.



The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting *Listeria monocytogenes* in all human food products and environmental samples (excluding samples from primary production).

Sample type	Enrichment
Meat, dairy, vegetables, seafood (except smoked fish and charcuteries)	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.
Smoked fish and charcuteries	Homogenize 25 g sample with 225 mL room temperature 24 LEB Complete media with buffer supplement. Incubate at 37°C for 24-28 hours.
Environmental samples	Swab a 4 x 4 in (10 x 10 cm) area with a sponge pre-moistened in D/E neutralizing broth. Homogenize 1 sponge with 90 mL room temperature 24 LEB Complete media. Incubate at 37°C for 24-28 hours.

2. Prepare BAX® System Lysis Reagent

Note: Do not over-mix the lysis reagent, as this can cause the mixture to foam.

To create a full bottle of BAX® System lysis reagent (prepares about 60 samples)

1. Add 150 µL of protease to one 12 mL bottle of lysis buffer.
2. Add 200 µL Lysing Agent 2 to the protease and lysis buffer mixture.
3. Cap the bottle and invert it several times to mix.

To create smaller volumes of BAX® System lysis reagent (prepares about 4 samples)

1. Add 12.5 µL protease to 1 mL of lysis buffer in a separate sterile container.
2. Add 16.5 µL Lysing Agent 2 to the protease and lysis buffer mixture.
3. Seal the container and invert it several times to mix.

3. Perform Sample Lysis

1. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C.
If you find that they have NOT been adequately chilled, call for technical assistance.
2. Create a rack file according to the instructions found under “Creating a Rack File”.
3. Turn on the Automated Thermal Block and select the RT Listeria program.

Note: Lysis may also be performed using separate analog heating and cooling blocks. See “Using Analog Heating and Cooling Blocks with the BAX® System Assays” for details and protocols.

4. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
5. Transfer 200 µL lysis reagent to each of the cluster tubes.
6. Transfer 5 µL enriched sample to the corresponding cluster tubes, using a new pipette tip for each sample.

Note: Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.

3. Perform Sample Lysis (Continued)

7. After all transfers have been completed, secure the caps.
8. After the “Load Samples” prompt appears on the Automated Thermal Block, place the rack of cluster tubes onto the Automated Thermal Block.

Press the **SELECT/CONTINUE** button to begin automated lysis.

Note: This process takes about 1 hour to complete.

9. When sample lysis is complete, the Automated Thermal Block displays the message “Sample PCR Ready” and sounds four audible beeps.

4. Hydrate PCR Tablets

1. Select a chilled (2-8°C) PCR cooling block.

Note: Remember that you must finish using the cooling block within 30 minutes.

2. Place a 32-well PCR tube rack over the insert.
3. Arrange strips of PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
4. Remove the aluminum block with the cluster tubes of sample lysate from the Automated Thermal Block and place it next to the PCR cooling block. Press the **SELECT/CONTINUE** button on the Automated Thermal Block to complete the program.
5. Remove the caps from the first strip of PCR tubes with the decapping tool.
6. Transfer 50 µL lysate into PCR tubes, then seal with flat optical caps.
7. Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated and sealed.

III. The BAX® System X5 PCR Assays

Listeria monocytogenes Assay

5. Complete a BAX® System Process Run

Take the rack of PCR tubes in the cooling block to the BAX® System X5 instrument.

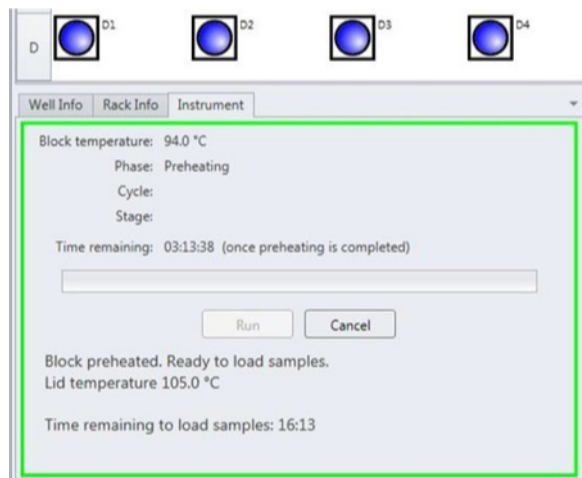
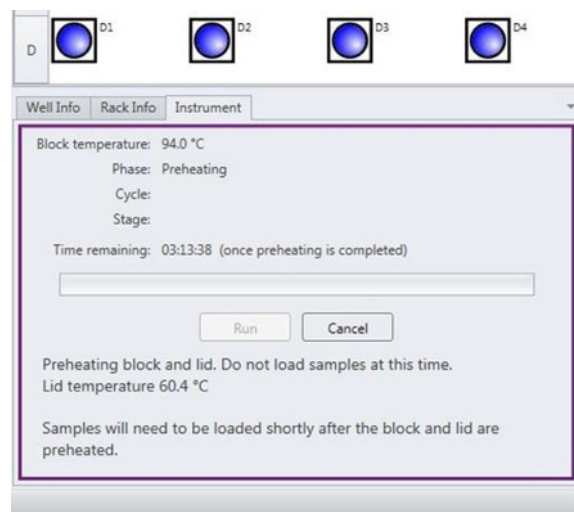
In the BAX® System X5 software window, select the Info Tab labeled “Instrument” to view the current instrument status.

Click **RUN** to begin pre-heating the instrument.

Note: If you have not created and saved a rack file, you will be prompted to do so before the instrument begins pre-heating.

Note: Samples must be loaded within 20 minutes after the RUN button is clicked. If samples are not loaded in 20 minutes, the process run times out and you will need to re-start the process.

When the instrument is heated, the instrument lid LED will turn green and the instrument prompt under the status bar reads “Block preheated. Ready to load samples.”

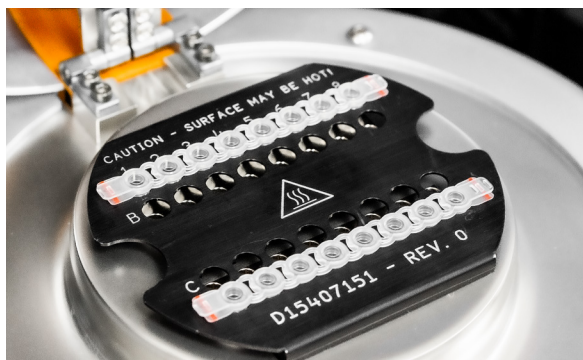


Remove the sample carrier with PCR tubes from the cooling block. Visually check PCR tubes to ensure the liquid contains no air bubbles and tubes are clean.

- IMPORTANT:** If air bubbles are present in the liquid, gently tap or flick the tubes to allow all air bubbles to escape.
- If the outside of the PCR tubes have dust, oils or residue, clean the outside of each PCR tube with a clean lab wipe.

5. Complete a BAX® System Process Run (Continued)

Open the BAX® System X5 instrument lid and load the PCR tubes into the instrument mount according to the setup of your rack file.



To process fewer than 32 samples, load samples first in Row A and Row D, starting at the corners.

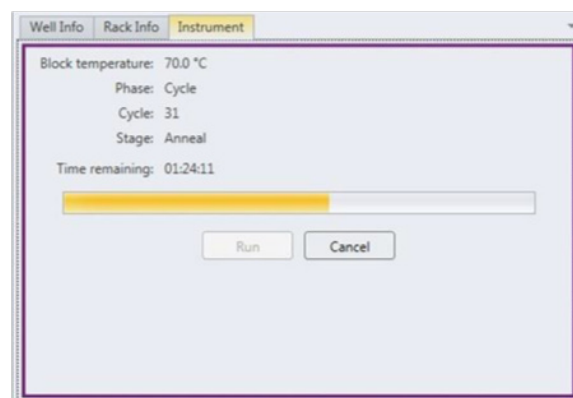
Once the two outer rows are full, then use Row B and Row C to load samples in a symmetric pattern to ensure the instrument mount remains balanced.

Close the instrument lid as soon as possible to prevent the instrument from cooling.

The process run begins automatically.

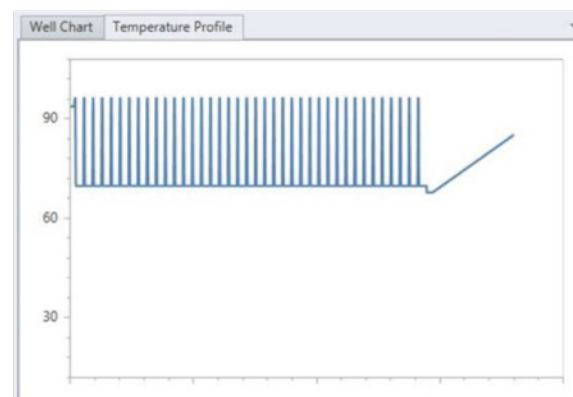
Note: After the instrument lid is closed, it may take a few moments for the instrument to stabilize before the run begins. When the instrument is stabilized, the process run begins automatically.

As the run progresses, the Instrument tab displays a status bar and estimates the time remaining before results can be viewed.



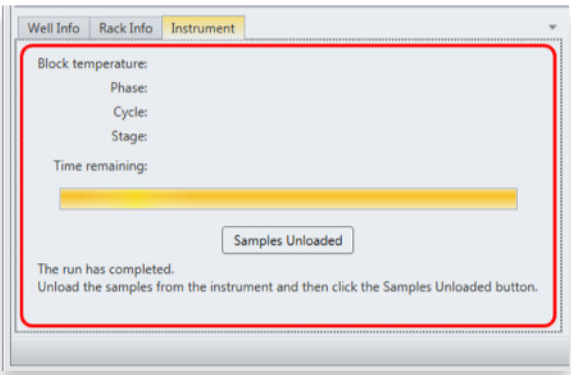
During the process, the instrument temperature is displayed graphically in the Graph Tab labeled "Temperature Profile".

Note: Variations in the temperature profile between different process runs are normal and do not affect results.



5. Complete a BAX® System Process Run (Continued)

After processing is complete, the instrument prompt under the status bar reads “The run has completed”.



Open the instrument lid and remove the PCR tubes from the instrument mount. Close the instrument lid.

Click the **SAMPLES UNLOADED** button to view results.

The temperature profile disappears after run is complete.

6. Review Results

Note: For more information about reading BAX® System results, see “Reviewing BAX® System Results”.

When processing in the BAX® System X5 instrument is complete, a new window appears showing each color-coded well with an icon in the center to illustrate the result:

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

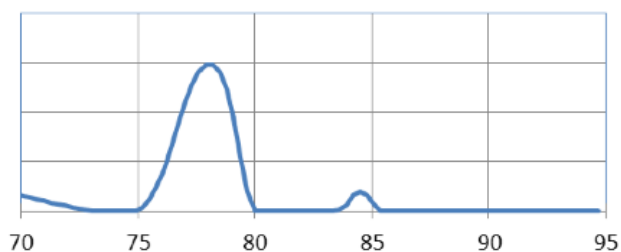
Positive *L. monocytogenes* Melting Curve Profiles

Samples that are positive for *L. monocytogenes* will display one target peak.

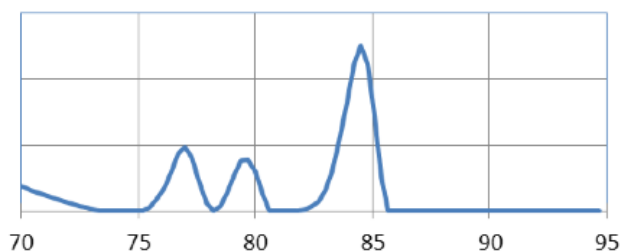
This peak usually appears between 83- 88°C.

The graph also displays one INPC, or control peak, which usually appears between 76-81°C. Often, the control peak appears as a “double-peak” in the presence of *L. monocytogenes*. If a sample displays a strong positive result, the INPC may be very small in comparison to the target peak.

Examples of Typical Melt Curves for Positive *L. monocytogenes* Samples



Weak X5 *L. monocytogenes* positive



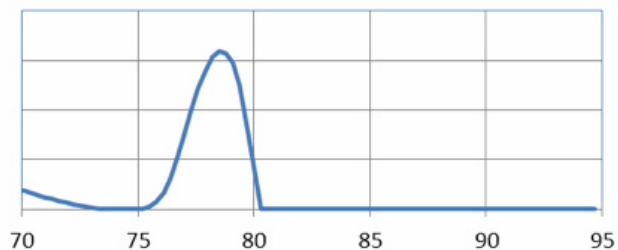
Strong X5 *L. monocytogenes* positive
with double control peak

Negative *L. monocytogenes* Melting Curve Profile

Samples that are negative for *L. monocytogenes* will display no target peaks.

The graph displays only the INPC, or control peak, which usually appears between 76-81°C.

Example of Typical Melt Curve for Negative *L. monocytogenes* Sample



7. Confirm Positive Results

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Using the *Salmonella* Assay

1. Enrich Samples

Assay Kit #KIT2025



The **AOAC Research Institute** has certified the BAX® System for detecting *Salmonella* in meat, poultry, fruit and vegetable products, dairy products, chocolate/bakery products, pasta, dry pet food and environmental.

This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

Sample type	Primary Enrichment	Secondary Enrichment
BAX® System MP media		
Ground beef	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.	None
Beef trim	Gently massage 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.	None
Spinach and lettuce	Combine 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media and swirl to soak entire sample. Incubate at 42°C for 8-24 hours.	None
Standard Media		
Meat and poultry, including raw ground beef	Homogenize (if ground or processed) or gently massage (if not ground) 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours.	None
Raw ground beef	Homogenize 25 g sample with 75 mL room temperature mTSB. Incubate at 42°C for 20-24 hours.	None
Frankfurters, chipped ham and cooked chicken	Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	None
Milk chocolate	Homogenize 25 g sample with 225 mL reconstituted nonfat dry milk. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Add 0.45 mL 1% brilliant green dye solution. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Nonfat dry milk	Pour 25 g sample slowly over 225 mL Brilliant Green Water (2 mL 1% brilliant green dye solution/L deionized water). Let stand at room temperature for 55-65 minutes. Do not mix or adjust pH. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Black pepper	Homogenize 25 g sample with 225 mL TSB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Peanut butter	Blend 25 g sample with 225 mL LB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2 if necessary. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Alfalfa sprouts	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BPW with 20 mg/L novobiocin. Incubate at 42°C for 20-24 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Raw frozen fish	Homogenize 25 g sample with 225 mL LB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours.	None
Orange juice	Homogenize 25 g sample with 225 mL Universal Pre-enrichment broth. Let stand at room temperature 55-65 minutes. Do not adjust pH. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

1. Enrich Samples (Continued)

Sample type	Primary Enrichment	Secondary Enrichment
Custard, 2% milk, chilled ready meal, cooked fish, prawns, macaroni, pizza dough, frozen peas, cream cheese, and dry pet food	Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Liquid eggs	Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Soy protein flour	Pour 25 g sample slowly over 225 mL LB in a flask. Let stand for 55-65 minutes at room temperature. Do not mix or adjust pH. Cap loosely. Incubate at 37°C for 18-22 hours.	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
Environmental sponges	<p>Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with 10 mL D/E Neutralizing Broth or equivalent.</p> <p>Finished Product Areas – Homogenize sponge with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8±0.02. Incubate at 35°C for 22-26 hours.</p> <p>Raw Materials Areas – Homogenize sponge with 225 mL pre-warmed (35°C) BPW. Let stand at room temperature for 55-65 minutes. Incubate at 35°C for 22-26 hours.</p>	Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

The **USDA National Poultry Improvement Plan (USDA-NPIP)** has developed an alternative protocol for detecting *Salmonella* in environmental samples, including drag swabs, fecal samples, dust, chick pads and meconium. Contact Hygiena Diagnostics Support for details and protocols.

The **U.S. Food and Drug Administration (US-FDA) Egg Safety Action Plan** has determined the BAX® System to be equivalent to the FDA-BAM method (Chapter 5: *Salmonella*) for detecting *Salmonella* Enteritidis in pooled liquid eggs, without the 96-hour hold time recommended by the BAM. See the FDA Testing methodology for *Salmonella* Enteritidis (SE) for details or contact Hygiena Diagnostics Support for protocols.

1. Enrich Samples (Continued)



QUA 18/03-11/02
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
<http://nf-validation.afnor.org>

The **French Association of Normalization (AFNOR Certification)** has certified the BAX® System according to the NF VALIDATION rules for detecting *Salmonella* in all human food products, animal feed and environmental samples (except primary production stage environmental samples).

Sample type	Primary Enrichment	Secondary Enrichment
Raw meats and poultry (without spices)	Homogenize 25 g sample with 225 mL pre-warmed (37°C) BPW. Incubate at 37°C for 16-20 hours.	None
Dairy (except milk powders)	Homogenize 25 g sample with 225 mL room temperature BPW supplemented with 20 mg/L novobiocin. Incubate at 42°C for 20-24 hours.	None
Other foods and environmental samples including composite foods, vegetables, seafood, animal feed and egg products	Homogenize 25 g sample with 225 mL room temperature BPW. Incubate at 37°C for 16-20 hours.	Transfer 10 µL enriched sample to 500 µL BHI. Incubate at 37°C for 3-4 hours.
Raw beef meat (including seasoned and frozen) in MP media	Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.	None
Other raw meat (including seasoned)	Homogenize 25 g sample with 225 mL BAX® System MP media. Incubate at 42°C for 24 hours.	None

2. Prepare BAX® System Lysis Reagent

Note: Do not over-mix the lysis reagent, as this can cause the mixture to foam.

To create a full bottle of BAX® System lysis reagent (prepares about 60 samples)

1. Add 150 µL of protease to one 12 mL bottle of lysis buffer.
2. Cap the bottle and invert it several times to mix.

To create smaller volumes of BAX® System lysis reagent (prepares about 4 samples)

1. Add 12.5 µL protease to 1 mL of lysis buffer in a separate sterile container.
2. Seal the container and invert it several times to mix.

3. Perform Sample Lysis

1. Make sure that the cooling blocks have been refrigerated or otherwise chilled to 2-8°C. If you find that they have NOT been adequately chilled, call for technical assistance.
2. Create a rack file according to the instructions found under “Creating a Rack File”.
3. Turn on the Automated Thermal Block and select the Gram Negative program.

Note: Lysis may also be performed using separate analog heating and cooling blocks. See “Using Analog Heating and Cooling Blocks with the BAX® System Assays” for details and protocols.

4. Break cluster tubes apart. Label and arrange cluster tubes in the rack according to your rack file.
5. Transfer 200 µL lysis reagent to each of the cluster tubes.
6. Transfer 5 µL enriched sample to the corresponding cluster tubes, using a new pipette tip for each sample.

Note: Do not shake or mix the enrichment before transferring samples to cluster tubes. If samples have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.

7. After all transfers have been completed, secure the caps.
8. After the “Load Samples” prompt appears on the Automated Thermal Block, place the rack of cluster tubes on the Automated Thermal Block.
9. Press the **SELECT/CONTINUE** button to begin automated lysis.

Note: This process takes about 1 hour to complete.

10. When sample lysis is complete, the Automated Thermal Block displays the message “Sample PCR Ready” and sounds four audible beeps.

4. Hydrate PCR Tablets

1. Select a chilled (2-8°C) PCR cooling block.

Note: Remember that you must finish using the cooling block within 30 minutes.

2. Place a 32-well PCR tube rack over the insert.
3. Arrange strips of PCR tubes according to your rack file. Mark the top of each strip to maintain orientation when placing strips in the instrument.
4. Remove the aluminum block with the cluster tubes of sample lysate from the Automated Thermal Block and place it next to the PCR cooling block. Press the **SELECT/CONTINUE** button on the Automated Thermal Block to complete the program.
5. Remove the caps from the first strip of PCR tubes with the decapping tool.
6. Transfer 50 µL lysate into PCR tubes, then seal with flat optical caps.
7. Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated and sealed.

5. Complete a BAX® System Process Run

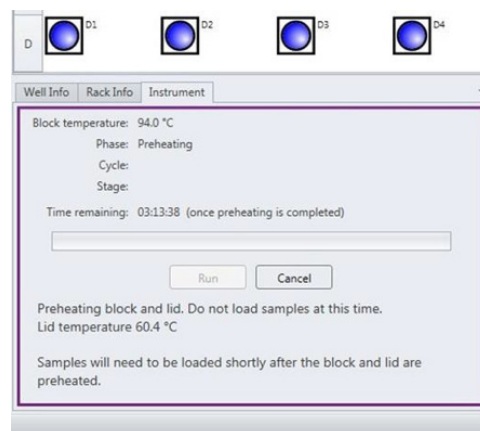
Take the rack of PCR tubes in the cooling block to the BAX® System X5 instrument.

In the BAX® System X5 software window, select the Info Tab labeled “Instrument” to view the current instrument status.

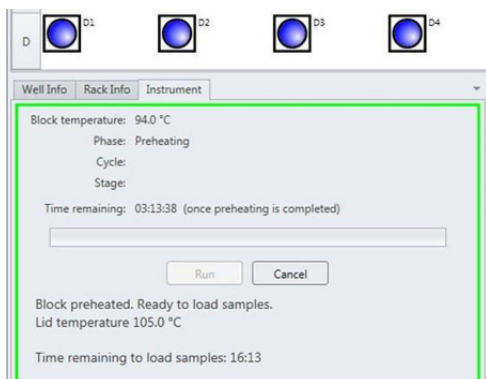
Click **RUN** to begin pre-heating the instrument.

Note: If you have not created and saved a rack file, you will be prompted to do so before the instrument begins pre-heating.

Note: Samples must be loaded within 20 minutes after the RUN button is clicked. If samples are not loaded in 20 minutes, the process run times out and you will need to re-start the process.



When the instrument is heated, the instrument lid LED will turn green and the instrument prompt under the status bar reads “Block preheated. Ready to load samples.”



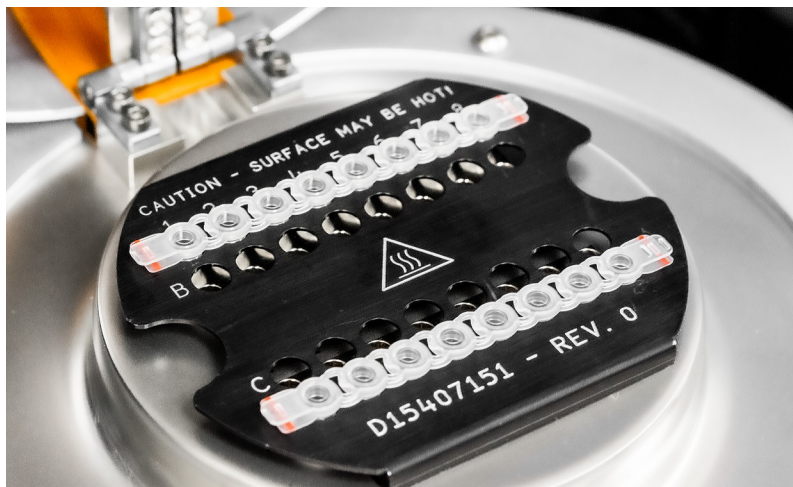
Remove the tube rack with PCR tubes from the cooling block. Visually check PCR tubes to ensure the liquid contains no air bubbles and tubes are clean.

- IMPORTANT:** If air bubbles are present in the liquid, gently tap or flick the tubes to allow all air bubbles to escape.
- If the outside of the PCR tubes have dust, oils or residue, clean the outside of each PCR tube with a clean lab wipe.

Open the BAX® System X5 instrument lid and load the PCR tubes into the instrument mount according to the setup of your rack file.

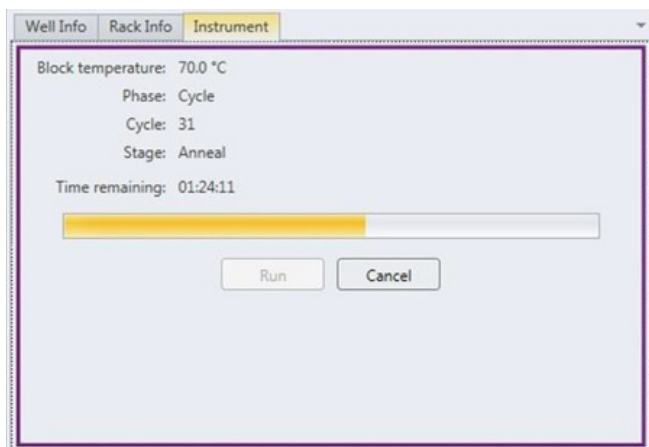
5. Complete a BAX® System Process Run (Continued)

To process fewer than 32 samples, load samples first in Row A and Row D, starting at the corners.



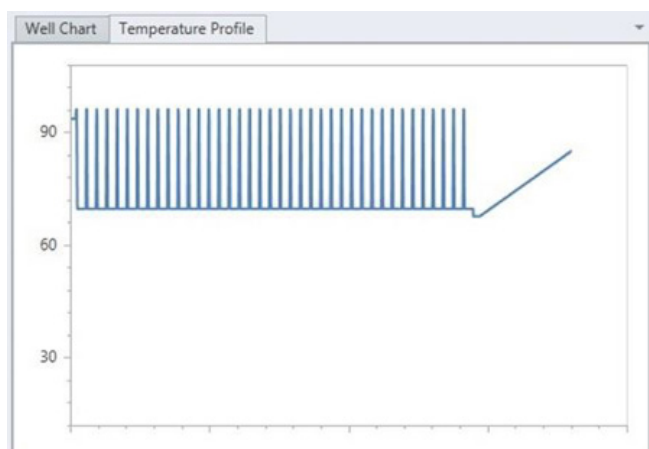
Once the two outer rows are full, then use Row B and Row C to load samples in a symmetric pattern to ensure the instrument mount remains balanced.

Close the instrument lid as soon as possible to prevent the instrument from cooling. The process run begins automatically.



Note: After the instrument lid is closed, it may take a few moments for the instrument to stabilize before the run begins. When the instrument is stabilized, the process run begins automatically.

As the run progresses, the Instrument tab displays a status bar and estimates the time remaining before results can be viewed.



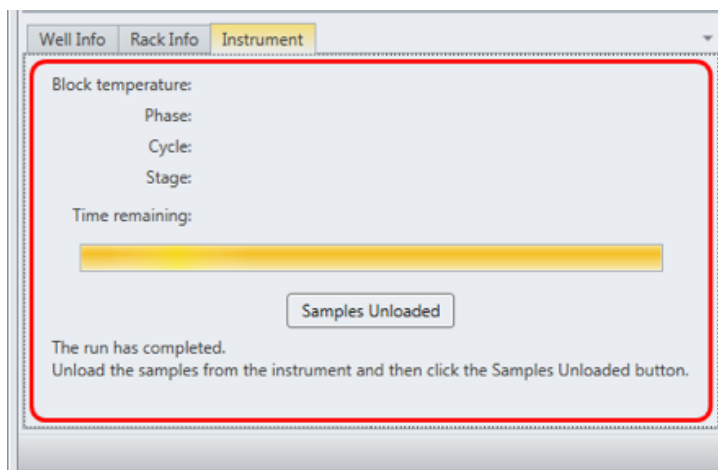
During the process, the instrument temperature is displayed graphically in the Graph Tab labeled "Temperature Profile".

Note: Variations in the temperature profile between different process runs are normal and do not affect results

5. Complete a BAX® System Process Run (Continued)

After processing is complete, the instrument prompt under the status bar reads “The run has completed”.

Open the instrument lid and remove the PCR tubes from the instrument mount. Close the instrument lid.




Click the **SAMPLES UNLOADED** button to view results. The temperature profile disappears after run is complete.

6. Review Results

Note: For more information about reading BAX® System results, see “Reviewing BAX® System Results”.

When processing in the BAX® System X5 instrument is complete, a new window appears showing each color-coded well with an icon in the center to illustrate the result:

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

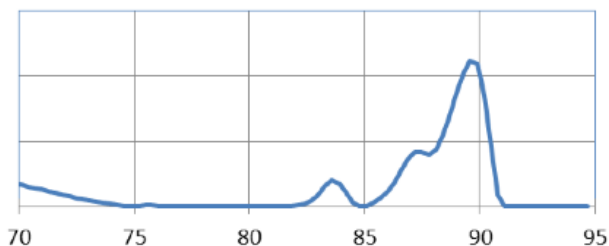
Positive *Salmonella* Melting Curve Profiles

Samples that are positive for *Salmonella* will display three target peaks.

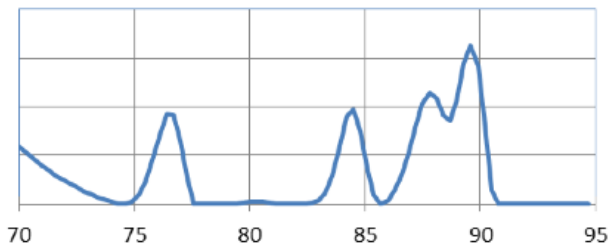
These target peaks usually appear between 82-91°C, with approximately 5°C distance between the first and the third target peak. If a sample displays a strong positive result, the target peaks may merge so that you see only two distinct peaks.

The graph also displays one INPC, or control peak, which usually appears between 76-80°C. If a sample displays a strong positive result, the INPC may be very small in comparison to the target peaks.

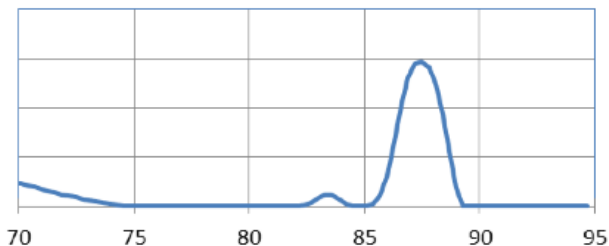
Examples of Typical Melt Curves for Positive *Salmonella* Samples



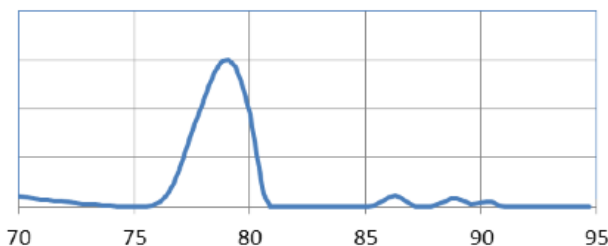
Strong X5 *Salmonella* positive with merged peaks 2 & 3



Moderate X5 *Salmonella* positive (notice the increased height of the control peak)



Strong X5 *Salmonella* positive



Weak X5 *Salmonella* positive

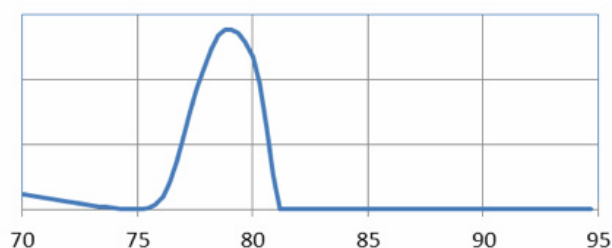
Negative *Salmonella* Melting Curve Profiles

Samples that are negative for *Salmonella* will display no target peaks. The graph displays only the INPC, or control peak, which usually appears between 76-80°C.

The graph also displays one INPC, or control peak, which usually appears between 79-82°C.

If a large target peak is also present, the INPC may be very small in comparison to the target peak.

Example of Typical Melt Curves for Negative *Salmonella* Samples



7. Confirm Positive Results

Method approved by AOAC

Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS method, see the protocol described in the Microbiology Laboratory Guidebook.

Method approved by AFNOR Certification

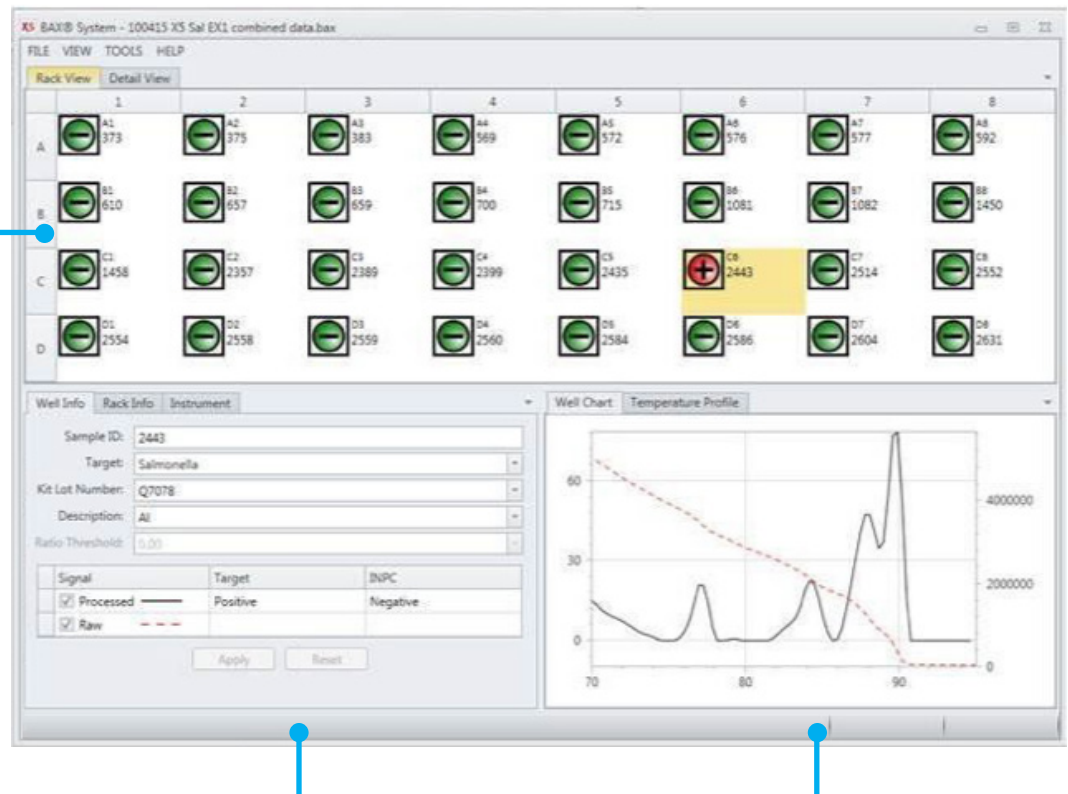
In the context of NF VALIDATION, samples identified as positive by the BAX® System must be confirmed by one of the following means:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Use any test method that has been certified according to the NF VALIDATION rules. The protocol of the certified test method must use the same preparation (enrichment, medium, etc.) as the BAX® System protocol and be followed in its entirety when used for confirmation.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Reviewing BAX® System Results

After you click on the **SAMPLES UNLOADED** button, a new window displays a modified rack view. BAX® System results are displayed as clear “yes” or “no” icons that do not require expert interpretation. Each well now appears in different colors with a symbol in the center to illustrate the result.



Rack View tab displays clear results icons – click on a sample well to view detailed information below

Well Info tab displays information for the selected sample

Well Chart tab displays a graph of the signal for the selected sample



Green (-)

=

Negative result



Red (+)

=

Positive result



Yellow (?)

=

Indeterminate result



Yellow (?) with red slash

=

Signal error

Reviewing Results Graphs Well Chart

Target peaks – Positive reactions show target peaks within a specified temperature range. Peaks can shift within their temperature range. In strong positive reactions, the peaks can shift to slightly lower temperatures, and in weaker reactions, the positives can shift to higher temperatures.

Control (INPC) peaks – INPC peaks occur in a specific temperature range for each target. Peaks vary in height depending on the presence or absence of the target. At high target levels, the control peak may be very small or absent.

Strength of positive result – The strength of a positive result is determined by comparing the peak heights of the target to the control. Samples with high target levels will display a strong positive result as a tall target peak and shorter control peak. In reactions with lower target levels, the control peak grows taller as the target peak gets shorter. When the target peak displays at less than 1/10 the height of the control peak, the positive result is considered weak.

Best Practices for Sample Enrichment, Preparation and Storage

Tips for Sample Prep Technique

- Always use new pipette tips when transferring samples or hydrating tablets. When dispensing lysis reagent, you can reuse pipette tips.
- Change gloves between each work step. Always use powder-free gloves.
- Use tweezers when removing cluster tubes caps and optical caps from the bag. Once the bag has been opened, store unused caps in a re-sealable container with a lid.
- Do not re-use any caps on cluster tubes or PCR tubes.
- Do not remove cooling block inserts from the refrigerator/freezer until they are needed. Finish using the cooling blocks within 30 minutes of removing inserts from the refrigerator/freezer.
- Use pre-sterilized cluster tubes for all lysis steps. Avoid using bulk cluster tubes that must be autoclaved before use.
- Break cluster tubes apart before use to help prevent cross-contamination between samples during transfers.
- When transferring samples, begin and complete one column at a time as shown in the BAX® System Ready Reference. Cap tubes after finishing a single row of samples to avoid cross-contamination.
- When transferring enriched samples to lysis tubes, check your technique for the following good practices to avoid sporadic indeterminate results:
 - Before transferring any enriched samples, check that all lysis tubes contain the same level of lysis reagent (200 µL).
 - Make sure to transfer the correct amount of enriched sample to the lysis tubes.
 - Slimy samples – Some samples become slimy after enrichment. When transferring these samples, insert just the very end of the pipette tip into the sample to avoid coating the entire tip. Wipe off any excess material by scraping the tip against the side of the sample container. In severe cases, you can use a clean wipe or tissue (fresh for each sample), but care must be taken to avoid cross-contamination.

Tips for Sample Prep Technique (Continued)

- Samples with particulates – Some samples contain particulate matter after enrichment. When transferring these samples, take care to pipette from the upper layer, not the bottom of the enrichment.
- Samples with oil/fat layers – Some samples generate an oil/fat layer on top of the enrichment. When transferring these samples, make sure to pipette from the aqueous layer below the oil/fat layer. Wipe off any coating of oil/fat from the tip as with slimy samples (above). If necessary, you can transfer an aliquot from the enrichment to a secondary tube to facilitate a clean transfer to lysis tubes.
- When transferring lysate to PCR tubes, verify the following:
 - Each PCR tube contains one PCR tablet.
 - The position of the PCR tubes in the rack matches the position in the rack file.
- Do not hydrate PCR tablets with lysate until the cycler has reached the correct load temperature and the “Ready for Rack Load” prompt appears.

Tips for Sample Preparation

- According to your laboratory SOP, prepare one positive control, one negative control and/or media blank and run along with your selected samples.
- After lysis has been completed, unopened lysate can be stored at 2-8°C for up to 7 days or at -20°C for up to 14 days before hydrating PCR tablets. Stopping the protocol prior to the end of lysis is not recommended, as this may compromise the results for some sample types.
- For re-testing purposes, enrichments can be stored at room temperature for 4 to 5 hours until results have been reviewed and validated. For longer storage (up to 48 hours) store at 2-8°C, unless validated otherwise internally.
- For confirmation purposes only, primary enrichments (before regrowth) could be stored at 2-8°C overnight or according to your laboratory SOP. However, these storage conditions have not been validated by Hygiena.
- Use a thermometer to ensure correct incubation temperature before preparing samples. Incubators should be within $\pm 2^{\circ}\text{C}$ of the specified temperature, unless you are following Health Canada and AFNOR Certifications standards which require an incubator capable of maintaining $\pm 1^{\circ}\text{C}$.

Tips for Enrichment Media

- When preparing enrichment broth, use deionized water that is compatible with PCR testing.
- Unless stated otherwise, all solutions should be pre-warmed to the specified temperature before use.
- Media should be warmed in a static incubator overnight. A hot water bath can also be used to warm media if a shorter pre-heating time is needed before preparing samples.
- Use a thermometer to ensure correct media temperature before preparing samples.

Note on Shaking Samples after Enrichment:

After enrichment, samples should not be shaken before aliquots are removed for lysis. For many sample types, shaking samples after enrichment increases the chance that larger particulates are transferred to the lysate, which could inhibit the PCR process. If samples have been agitated after enrichment, let sit for at least 10 minutes before transferring aliquots to cluster tubes for lysis.

Tips for Sample Lysis

- Use a thermometer to ensure set temperatures are correct and within the approved temperature stability ranges before heating samples (see “Note on Heating Block Temperature Stability” in Chapter I).
- When preparing lysis reagent, mix the protease and lysis buffer by capping the bottle and inverting 8-10 times. Do not over-mix lysis reagent, which can cause the mixture to foam.
- If desired, you can make smaller volumes of lysis reagent by keeping the ratio at 12.5 μ L protease to 1 mL lysis buffer. Lysis reagent in capped tubes will remain stable for up to two weeks when refrigerated at 2-8°C.
- For steps using a cooling block, minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible.
- Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.
- Unopened lysates may be stored for up to one week for later testing when refrigerated at 4°C.
- Opened lysates may be stored for up to one week for later testing when frozen at -20°C.

Tips for PCR Tablet Hydration

- Remove only the required number of PCR tubes from the bag in the refrigerator. Reseal the bag tightly.
- After placing PCR tubes in the holder, mark the top of each strip to maintain orientation when placing strips in the instrument.
- When hydrating tablets, only remove the caps from one strip of tubes at a time to avoid cross-contamination.
- Visually check that each PCR tube contains a white tablet. If tablets have shrunk or appear pink, discard tubes and replace with new ones before proceeding.
- PCR tablets must be hydrated and re-sealed within 10 minutes after loosening the caps from the PCR tubes.
- Secure flat optical caps on PCR tubes tightly after hydrating tablets, as loose caps can result in PCR failure or invalid detection.
- Keep samples chilled at 2-8°C until immediately before loading them into the instrument.

Note: After loading samples into the BAX® System instrument, store the racks of lysates at 2-8°C until you have finished reviewing the results, then dispose of the waste according to your site practices. Cooling blocks and inserts should be wiped with 10% bleach, rinsed with deionized water and blotted dry. Return the inserts to the refrigerator.

Tips for Interpretation of Melting Curves

- Only peaks in the expected locations should be used for interpretation. See the melting curve profiles at the end of each BAX® System assay protocol for details.
- A control peak should be visible at approximately 79°C for negative reactions. See the melting curve profiles at the end of each BAX® System assay protocol for details.

Note: The melting curves for negative *E. coli* O157:H7 reactions are slightly different.

- In a strong positive reaction, the control peak may be small or absent.
- Target peaks can shift within the specified temperature range. In strong positive reactions, the peaks may shift to slightly lower temperatures. In weaker positive reactions, the peaks may shift to slightly higher temperatures.

Using Analog Heating and Cooling Blocks with the BAX[®] System Assays

If you do not wish to use the Automated Thermal Block for automated sample lysis, the lysis steps for the BAX[®] System method may also be performed using separate analog heating and cooling blocks. Follow the instructions below to perform sample lysis using separate analog heating and cooling blocks.

1. Follow the protocols listed under “Enrich Samples” to combine samples with the appropriate enrichment media and incubate them at the appropriate temperature and duration.
2. Turn on the two analog heating blocks and set them to the appropriate temperatures for your target:

BAX [®] System X5 Assay	Set Heating Blocks To
<i>E. coli</i> O157:H7 <i>Salmonella</i>	37°C and 95°C
Genus <i>Listeria</i> <i>L. monocytogenes</i>	55°C and 95°C

3. Make sure that the two cooling blocks have been refrigerated overnight or otherwise chilled at 2-8°C.
4. Follow the instructions above to prepare BAX® System lysis reagent.
5. Transfer 200 µL lysis reagent to each of the cluster tubes.
6. Transfer 5 µL* enriched sample to the corresponding cluster tubes, using new pipette tips for each sample.

** For E. coli O157:H7, transfer 20 µL enriched sample if using BAX® System MP media for enrichment*

7. Place cluster tubes into the pre-warmed heating block (95°C) for 10 minutes.
8. After heat step, place in cooling block (2-8°C) for at least 5 minutes.
9. Remove the caps from the first strip of PCR tubes with the decapping tool and transfer 50 µL lysate into PCR tubes, then seal with flat optical caps.
10. Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated and sealed.
11. Follow the instructions in “Complete a BAX® System Process Run” under specific assay to complete the batch run.

Enrichment Media Recipes

Enrichment recipes are included in this section for your convenience only and are subject to change without notice. Always check the following sources or your reference manual of choice for the most current update:

- **USDA-FSIS Microbiology Laboratory Guidebook –**
<http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook>
- **FDA Bacteriological Analytical Manual –**
<http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm>
- **Health Canada Compendium of Analytical Methods**
<http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php>

Important Tips on Enrichment Media:

- Unless stated otherwise, all solutions should be pre-warmed to the specified enrichment temperature before use.
- Media should be warmed in a static incubator overnight. A hot water bath can also be used to warm media if a shorter pre-heating time is needed before preparing samples.
- Use a thermometer to ensure correct media temperature before preparing samples.

24 LEB media

Using 24 LEB Complete (blended media)

- Catalog MED2005 (Hygiena) or CM1154 (Oxoid) – 2.5 kg
- Completely dissolve 21.75 g 24 LEB Complete media in 500 mL distilled water. Autoclave at 121°C for 15 minutes, then cool to room temperature before use. Final pH 7.4±0.2 at 25°C.

Using 24 LEB Base and Selective Supplement

- 24 LEB Base – Catalog MED2004 (Hygiena) or CM1107 (Oxoid) – 500 g
- 24 LEB Selective Supplement – Catalog MED2015 (Hygiena) or SR0243E (Oxoid) – 10 vials
- Add 21.75 g 24 LEB base to 500 mL distilled water. Mix and autoclave at 121°C for 15 minutes. Cool mixture to ≤ 50°C. Hydrate 1 vial 24 LEB selective supplement with 5 mL sterile distilled water, mixing gently to dissolve. Add hydrated supplement to diluted base and mix thoroughly. Cool to 25-30°C before use. Final pH 7.4±0.2 at 25°C.

24 LEB media with buffer supplement

Required by NF VALIDATION for smoked fish and charcuteries

Note: This buffer supplement may also be beneficial for other samples with a low pH or those that experience a drop-in pH during enrichment. Before testing any food types not validated by AOAC or AFNOR Certification, it is strongly recommended that you internally validate samples with this assay to determine if the buffer supplement is required.

- 24 LEB media - see above
- 24 LEB Buffer Supplement – Catalog MED2000 (Hygiena) or BO1204 (Oxoid)
- Prepare 24 LEB media as described above. Add 10 mL pre-warmed (37°C) buffer supplement to each 225-mL volume of 24 LEB enrichment broth and mix. Cool to 25-30°C before use. Final pH 7.4 ± 0.2 at 25°C.

BAX® System MP media

(Previously BAX® System *E. coli* O157:H7 MP media broth)

StatMedia™ soluble packet

- BAX® System MP media – Catalog No. MED2016 – 33.75 g use 1.5 L sterile deionized water
- Add pre-warmed (42°C) sterile water to sterile container. Add soluble packet to container and mix until dissolved. Final pH, 7.2 ± 0.2 . Autoclaving is not required if used within 3-4 hours. For longer storage, autoclave at 121°C for 15 minutes, then store at 2-8°C for up to two weeks.

Bulk powder

- BAX® System MP media – Catalog No. MED2003 – 2.5 kg
- Dissolve 22.5 g in 1 L deionized water. Adjust pH to 7.2 ± 0.2 at 25°C, then autoclave at 121°C for 15 minutes.

BAX® System *Listeria* broth

- BAX® System media for *Listeria* – Catalog No. MED2002 – 2.5 kg
- Dissolve 60.4 g in 1 L deionized water. Adjust pH to 7.2 ± 0.2 at 25°C, then autoclave at 121°C for 15 minutes.



CAUTION: Do not boil the media or overheat the media in the autoclave.

Do not extend autoclaving beyond 15 minutes.

III. The BAX® System X5 PCR Assays

Enrichment Media Recipes

Brain heart infusion (BHI) broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Calf brain, infusion from 200 g	7.7 g	Dissolve ingredients in deionized water with gentle heat. Autoclave at 121°C for 15 minutes.
Beef heart, infusion from 250 g	9.8 g	
Proteose peptone (Difco) or polypeptone (Bioquest)	10 g	
NaCl	5 g	
Na ₂ HPO ₄	2.5 g	
Dextrose	2.0 g	
Deionized water	1 L	

Note: Commercial preparations of BHI broth are available from Oxoid (catalog #CM1032 for FDA-BAM formulation or #CM1135 for AFNOR Certification formulation) and other distributors.

Brilliant green dye solution (1%)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Brilliant green dye	1 g	Dissolve dye in 10 mL deionized water, then dilute to 100 mL. Before use, test all batches of dye for toxicity with known positive and negative test microorganisms.
Deionized water	10 mL	

Buffered *Listeria* enrichment broth (BLEB)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
TSB	30 g	Dissolve ingredients without the 3 selective agents in deionized water. Autoclave at 121°C for 15 minutes.
Yeast extract	6 g	
Monopotassium phosphate	1.35 g	Note: Optionally a filter-sterilized 10% (w/v) sodium pyruvate solution may be added after autoclaving.
Disodium phosphate (anhydrous)	9.6 g	
Sodium Pyruvate (sodium salt) (Sigma)	1.11 g	Prepare acriflavine and nalidixic acid supplements as 0.5% (w/v) stock solutions in deionized water. Prepare cycloheximide supplement as 1.0% (w/v) stock solution in 40% (v/v) solution of ethanol in water. Filter-sterilize the three stock solutions and store at 4°C.
Deionized water	1 L	
Cycloheximide	50 mg/L	Aseptically add 0.455 mL acriflavine stock solution, 1.8 mL nalidixic acid stock solution and 1.15 mL cycloheximide stock solution to 225 mL enrichment after 4-hour incubation at 30°C.
Acriflavine HCl	10 mg/L	
Nalidixic acid	40 mg/L	

Note: Commercial preparations available from Oxoid (BLEB #CM0897 and supplement #SR0141).

Buffered peptone water

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Peptone	10 g	Dissolve ingredients in deionized water. Autoclave at 121°C for 15 minutes. Final pH 7.2±0.2.
Sodium chloride	5 g	
Disodium phosphate	3.5 g	
Mono-potassium phosphate	1.5 g	
Deionized water	1 L	

Note: Commercial preparations are available from Hygiena (Part No. MED2010 [D15452608]) 2.5 Kg or (Part No. MED2011[D15452596]) 500 g or from Oxoid (#CM0509).

III. The BAX® System X5 PCR Assays

Enrichment Media Recipes

Buffered peptone water with novobiocin

Mix ingredients as above without antibiotic, then autoclave at 121°C for 15 minutes. Prepare novobiocin as a 20 mg/mL solution in deionized water. Filter sterilize through a 0.2 µm filter, then transfer 1 mL novobiocin solution to 1 L of the cooled (< 50°C) buffered peptone water broth. Final pH, 7.2±0.2. Use broth within 24 hours of adding the antibiotic.

Complete selective enrichment broth

Ingredients	Amount	Instructions
Tryptone soya broth powder	30 g	Dissolve ingredients without antibiotics in deionized water, adjust pH to 7.3, then autoclave at 121°C for 15 minutes.
Yeast extract	6 g	
Deionized water	1 L	Prepare acriflavine and nalidixic acid supplements as 0.5% (w/v) stock solutions in deionized water. Prepare cycloheximide supplement as 1.0% (w/v) stock solution in 40% (v/v) solution of ethanol in water. Filter-sterilize the three stock solutions and store at 4°C.
Cycloheximide	50 mg	
Acriflavine HCl	10 mg	
Nalidixic acid	40 mg	Add stock solutions: 0.45 mL acriflavine, 1.8 mL nalidixic and 1.15 mL cycloheximide to 225 mL enrichment broth.

Demi-Fraser broth

Ingredients	Amount	Instructions
Demi-Fraser broth base (Difco #265320 or equivalent)	55 g	Mix base in deionized water, adjust pH to 7.2±0.2, then autoclave at 121°C for 15 minutes.
Deionized water	1 L	

Note: Ferric ammonium citrate is not required in this formulation.

Commercially available preparations are also available from Oxoid as Fraser broth base (#CM0895) and Half-Fraser Supplement (#SR0166).

Dey-Engley (D/E) Neutralizing Broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Tryptone	5 g	Dissolve all ingredients in deionized water. Autoclave at 121°C for 15 minutes. Final pH, 7.6±0.2.
Yeast extract	2.5 g	
Glucose	10 g	
Sodium thioglycollate	1 g	
Sodium thiosulfate	6 g	
Sodium bisulfite	2.5 g	
Polysorbate 80	5 g	
Lecithin (soybean)	7 g	
Bromocresol purple	0.02 g	
Deionized water	1 L	

Note: Commercial preparations of D/E neutralizing broth are available from Difco (#281910) and BBL (#298318).

Lactose broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Beef extract	3 g	Dispense 225 ml portions into 500 ml Erlenmeyer flasks. After autoclaving at 121°C for 15 minutes and just before use, aseptically adjust volume to 225 mL. Final pH, 6.9±0.2.
Peptone	5 g	
Lactose	5 g	
Deionized water	1 L	

III. The BAX® System X5 PCR Assays

Enrichment Media Recipes

MOPS-BLEB* broth

* 3-(N-Morpholino) propanesulfonic acid - buffered *Listeria* enrichment broth

Source: Silbernagel et al, *Journal of AOAC International* 87 (2) 395-410 (2004)

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Mix ingredients without antibiotics, adjust pH to 7.3±0.2, then autoclave at 121°C for 15 minutes.
MOPS free acid	6.7 g	
MOPS sodium salt	10.5 g	
Yeast	6 g	Prepare acriflavine and nalidixic acid supplements as 0.5% (w/v) stock solutions in deionized water. Prepare cycloheximide supplement as 1.0% (w/v) stock solution in 40% (v/v) solution of ethanol in water. Filter-sterilize the three stock solutions and store at 4°C.
Deionized sterile water	1 L	
Acriflavine	0.015 g	
Nalidixic acid	0.04 g	Add 3 mL acriflavine, 8 mL nalidixic and 5 mL cycloheximide to 1 L of <i>Listeria</i> enrichment broth.
Cyclohexamide	0.05 g	

Nonfat dry milk, reconstituted

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Nonfat dry milk	100 g	Suspend 100 g dehydrated nonfat dry milk in 1 liter deionized water. Swirl until dissolved. Autoclave at 121°C for 15 minutes.
Deionized water	1 L	

Trypticase soy broth (TSB)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Trypticase peptone	17 g	Dissolve ingredients with heat in deionized water. Dispense 225 mL into 500 mL Erlenmeyer flasks. Autoclave at 121°C for 15 minutes. Final pH 7.3±0.2. For trypticase soy broth without glucose, prepare as above, but omit 2.5 g glucose.
Phytone peptone	3 g	
NaCl	5 g	
K ₂ HPO ₄	2.5 g	
Glucose	2.5 g	
Deionized water	1 L	

Universal pre-enrichment broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Tryptone	5 g	Dissolve ingredients with heat in deionized water. Autoclave at 121°C for 15 minutes. Final pH, 6.3±0.2.
Proteose peptone	5 g	
KH ₂ PO ₄	15 g	
Na ₂ HPO ₄	7 g	
NaCl	5 g	
Dextrose	0.5 g	
MgSO ₄	0.25 g	
Ferric ammonium citrate	0.1 g	
Sodium pyruvate	0.2 g	
Deionized water	1 L	

III. The BAX® System X5 PCR Assays

Enrichment Media Recipes

UVM broth, modified

Source: USDA-FSIS Microbiology Laboratory Guidebook

Ingredients	Amount	Instructions
Proteose peptone	5 g	<p>Dissolve ingredients in deionized water. Autoclave at 121°C for 15 minutes.</p> <p>DO NOT OVERHEAT; COOL AT ONCE AFTER REMOVAL FROM THE STERILIZER. IF THE MEDIUM BLACKENS OR DARKENS, IT HAS BEEN OVERHEATED AND MUST BE DISCARDED.</p> <p>Store in the refrigerator.</p> <p>Final pH 7.2±0.2 at 25°C.</p>
Tryptone	5 g	
Lab Lemco powder (Oxoid)	5 g	
Yeast extract	5 g	
NaCl	20 g	
KH ₂ PO ₄	1.35 g	
Na ₂ HPO ₄	12 g	
Esculin	1 g	
Naladixic acid (2% in 0.1 M NaOH)	1.0 mL	
Acriflavine	12 mg	
Deionized water	1 L	

Note: Commercially available preparations are available from Oxoid as UVM Base (#CM0863) and Supplement (#SR0142).

Confirmation Utility

Note: *This procedure is not part of the methods approved by AOAC.*

Good laboratory practice suggests that you not use the same method to both screen a sample and confirm its results. When using the BAX® System to screen a sample, any desired confirmation is usually performed through biochemical methods. Conversely, when screening with traditional biochemical methods, you can use the BAX® System to identify suspect target colonies on selective agar plates.

The sample preparation steps vary slightly from those used with the screening protocol; otherwise, the method is the same. The only additional supplies you need are plastic disposable inoculating needles and sterile diluent, such as 0.1% peptone water.

To identify suspect colonies, follow these steps:

1. Examine selective agar plates for suspect colonies and identify those that require confirmation.
2. Prepare lysis tubes according to the screening protocol and fill out your sample tracking sheet.
3. Add 1 mL of sterile diluent to sterile tubes, and label them to correspond with your lysis tubes.

Note: *If confirming samples with the BAX® System real-time STEC suite (Screening, Panel 1 or Panel 2 assays), use TSB or mTSB as the sterile diluent.*

Note: *If your BAX® System results are positive, you can then use these tubes to streak plates for isolation and further identification of the target organism.*

4. Touch a plastic disposable needle to the agar plate to pick a suspect colony.

Note: *Avoid picking larger colonies, as adding too much sample DNA to the dilution tube may affect results.*

5. Dip the needle into the corresponding dilution tube and swirl the needle to release the colony. Remove the needle, then cap the tube and mix.
6. Uncap the dilution tube, transfer 5 µL of the cell suspension into the corresponding lysis tube containing 200 µL lysis reagent, and mix.

Note: *If confirming samples with the BAX® System real-time STEC suite (Screening, Panel 1 or Panel 2 assays), pipette 20 µL of the cell suspension into the corresponding lysis tube containing 200 µL lysis reagent.*

7. Repeat steps 4-6 for each of your suspect colonies, using a new needle for each pick.
8. Continue with the BAX® System testing protocol from “Perform Lysis.”

Atypical Colony Confirmation Results

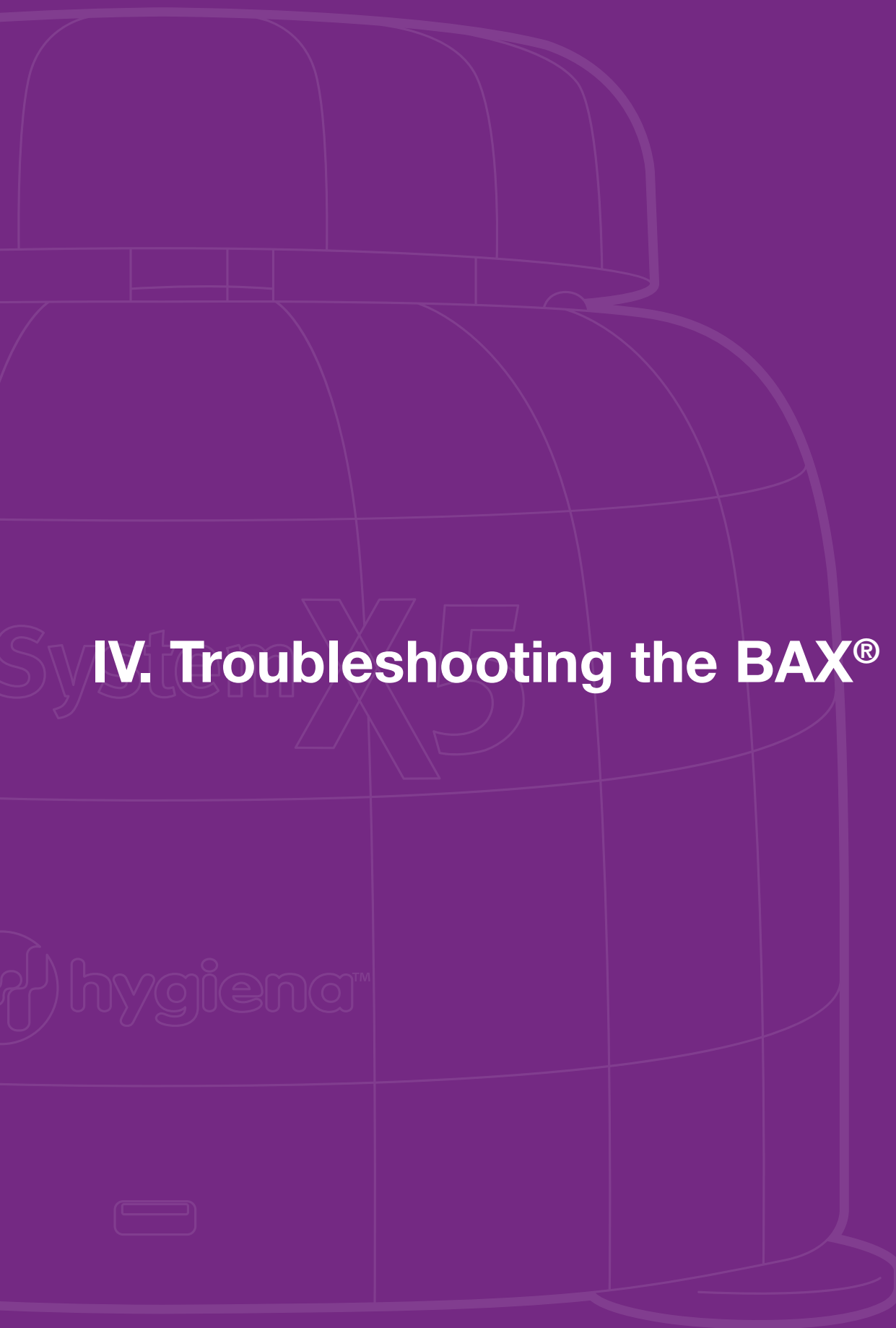
When using the BAX® System to confirm a suspect colony, it is expected that positive results will be strong due to the purity of the culture (see the melting curve profiles at the end of each BAX® System assay protocol for details). However, if an excessive amount of non-target DNA is present in the lysis sample, weak positive results may occur for the target organism. If this occurs, the resulting graphs will display a large INPC peak relative to the target peaks.

If you do not find a strong positive confirmation, follow these steps to verify atypical positive results from a colony confirmation:

1. Prepare an overnight enrichment from the suspect colony according to your lab's standard procedure.

Note: You can also re-grow 10 µL of solution from the previous confirmation dilution tube (step 3, above) overnight to serve as the enrichment.

2. Add 5 µL re-grown sample to 200 µL prepared lysis reagent.
3. Follow the appropriate BAX® System assay protocol (see Chapters II to V) to run a full process for the target organism.
4. View the displayed results. A positive result confirms the suspect colony for the target organism.



IV. Troubleshooting the BAX® System

hygiena™

IV. Troubleshooting the BAX® System

The troubleshooting tips provided in this chapter are intended to provide general guidance for the most common customer issues with the BAX® System X5 instrument and procedures.

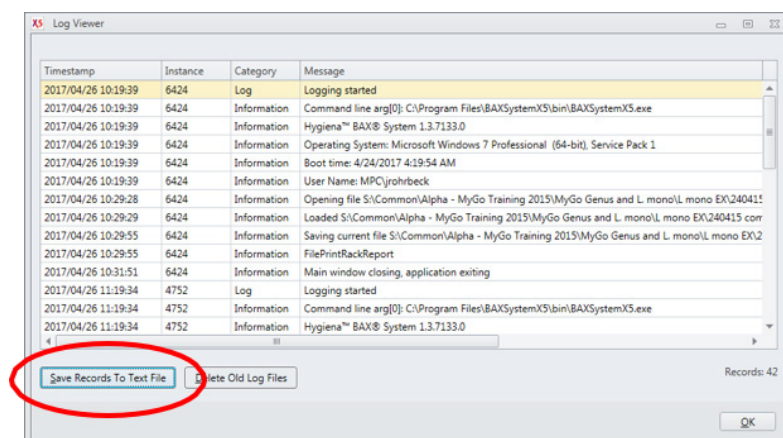
For additional technical assistance, contact Hygiena Diagnostics Support or your local distributor.

Accessing Log Files

Diagnostics Support may request log files during troubleshooting.

Follow these instructions to prepare the log file to send to Diagnostics Support

From the Tools menu of the BAX® System X5 software, select “Log Viewer...” to open the Log Viewer window.



At the bottom of the Log Viewer window, click the **SAVE RECORDS TO TEXT FILE** button to open the save dialogue.

Navigate to the location where you would like to save the file. Type a new name for the file, or you can keep the file name automatically generated by the BAX® System X5 software.

Click the **SAVE** button to save the log file.

Troubleshooting Indeterminate or Error Results

- Air bubbles in PCR tubes
- Missing PCR tubes
- Non-specific reactions
- Straight line signal

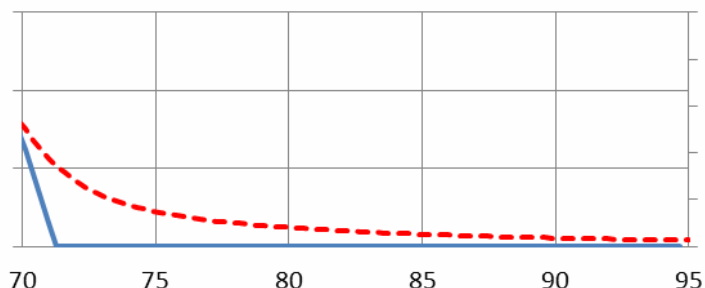
In these situations, you can examine the shape of the melt curve and its Y-axis scale to help diagnose the problem.

Air Bubbles in PCR Tubes

What It Looks Like – There appears to be no reaction within the sample data.

Note: In many cases, samples with air bubbles may not display indeterminate results; however, the signal peaks are shifted away from the expected temperature range or appear as “weak” results.

Cause – Air bubbles were present in the sample liquid before PCR tubes were loaded into the BAX® System X5 instrument. Air bubbles commonly occur when either air is trapped under the tablet and is not displaced by the sample fluid, or air is present in the pipette tip and added to the PCR tube with the sample liquid.



Action - Select OPERATION > REANALYZE WELLS and follow the screen prompts. The associated data will display as captured.

Missing PCR Tubes

What It Looks Like – The Raw data appears as a jagged pattern of multiple steps. The Processed view shows system noise at low signal levels.



Cause – PCR tubes were not placed in the rack according to your rack file layout. This happens most frequently when you are running a partial rack of samples and mistakenly place an entire strip of PCR tubes in the wrong column of the rack.

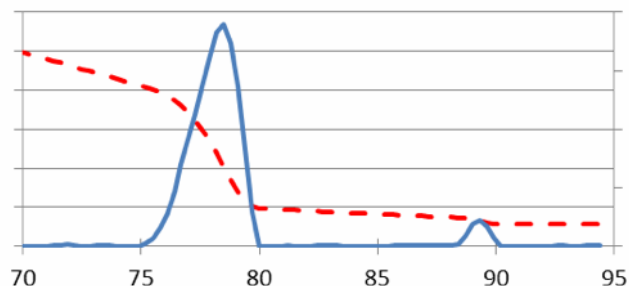
Action – Re-label the wells in your file according to the sample placement in the rack and assign the appropriate target. Select OPERATION > REANALYZE WELLS and follow the screen prompts. The associated data will display as captured.

Non-Specific Reactions

What It Looks Like – The Processed data shows small peaks in the 80-95°C range and broad peaks in the 88-95°C range. This view may also show subtle system noise.

Cause – Non-specific reactions may be caused by the following:

- Samples were not loaded into the BAX® System cycler immediately after hydrating PCR tablets.
- After lysis, samples were not maintained at 2-8°C before loading into the cycler.



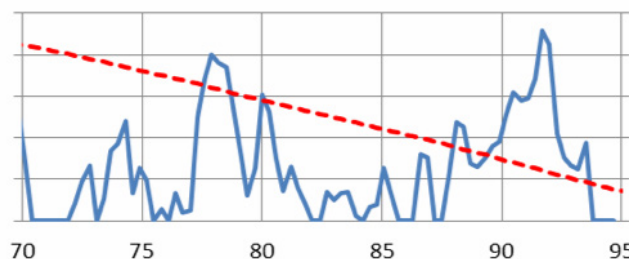
Action - Repeat the assay from last enrichment. Follow all steps of the protocol exactly as described in this User Guide or the assay's package insert.

Straight Line Signal

What It Looks Like – The Raw data appears as a negatively sloped line. The Processed data is blank or shows system noise.

Cause – Straight line signal may be caused by the following:

- PCR tablet was accidentally removed from the PCR tube before processing (missing tablet).
- Not enough sample lysate was added to the PCR tubes, resulting in a dry PCR tablet.
- Samples were run in “Detection only” mode instead of “Run Full Process” mode.
- Laboratory contaminants (such as powder from gloves, detergents, disinfectants, etc.) have interfered with the PCR process.



Action – Re-label the wells in your file according to the sample placement in the rack and assign the appropriate target. Select OPERATION > REANALYZE WELLS and follow the screen prompts. The associated data will display as captured.

Troubleshooting Process Interruptions

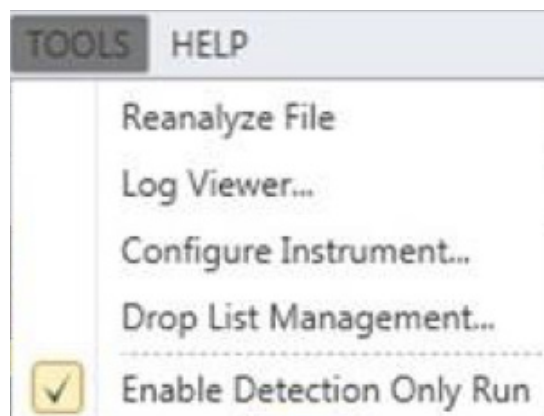
In some cases, a full process run in the BAX® System X5 instrument may be interrupted before results are displayed for such reasons as

- Unexpected power failure
- Data communication error
- Accidental cancellation of the process

In these cases, it may be possible to re-run the same PCR tubes in the instrument to generate results with the Detection Only operation. This option allows you to re-analyze samples that have already begun the detection phase of the standard full process. Results are available after about 1 hour.

Do not use the Detection Only process unless instructed to do so by Hygiena Diagnostics Support or your local Hygiena representative or distributor.

Note: The Detection Only option *SHOULD NOT* be used to re-analyze samples for which results have already been displayed.



Under the Tools menu, select “Enable Detection Only Run”.

Once selected, the “Enable Detection Only Run” option in the menu will be marked with a check.

Follow the instructions under “Complete a BAX® System Process Run” for the appropriate PCR assay to pre-heat the instrument, load samples and start processing.

IV. Troubleshooting the BAX® System

The Detection Only process begins with an initial hold step, followed by a melt step.

As the run progresses, the Instrument tab displays a status bar and estimates the time remaining before results can be viewed.

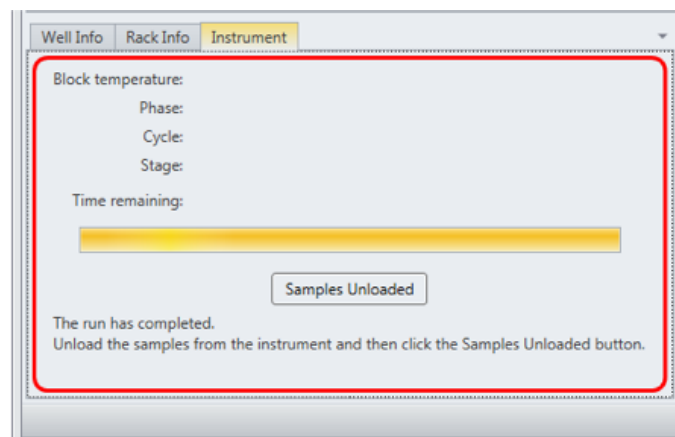
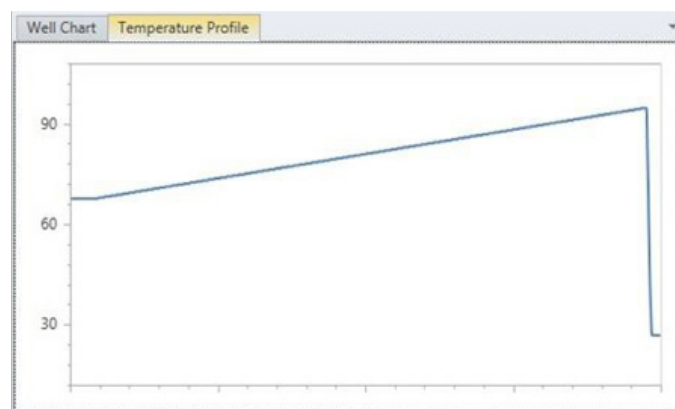
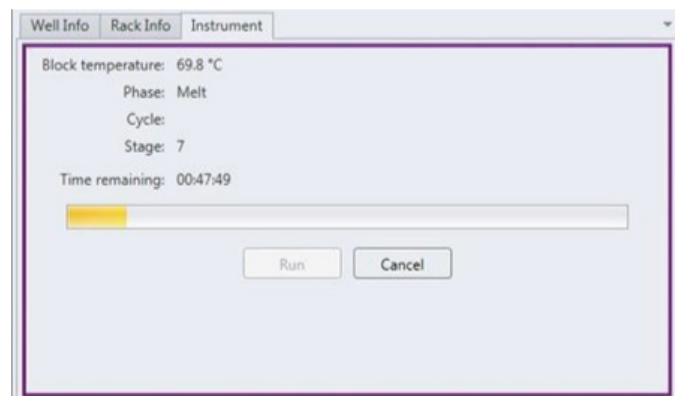
During the process, the instrument temperature is displayed graphically in the Graph Tab labeled “Temperature Profile”.

Note: Variations in the temperature profile between different process runs are normal and do not affect results.

After processing is complete, the instrument prompt under the status bar reads “The run has completed”.

Open the instrument lid and remove the PCR tubes from the instrument mount. Close the instrument lid. Click the **SAMPLES UNLOADED** button to view results.

The temperature profile disappears after run is complete.





V. BAX® SYSTEM X5 LIMITATION OF WARRANTY AND LIABILITY

 **hygiena™**

V. BAX® SYSTEM X5 LIMITATION OF WARRANTY AND LIABILITY

BAX® SYSTEM X5 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.

1. BAX® System X5 Equipment should only be used with BAX® System X5 assays.
2. When used with BAX® System X5 PCR assays, BAX® System X5 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 X5 PCR 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, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING. User assumes all risk and liability resulting from use of the BAX® System X5 Equipment, PCR assays and media, whether used singly or in combination with other products.
3. BAX® System software: Hygiena warrants that for a period of 60 days from the date of first date of use by the Customer/end user, BAX® System software media will be free from defect in materials and workmanship and that the BAX® System software will substantially perform in accordance with the accompanying BAX® System software documentation. EXCEPT FOR THE EXPRESS WARRANTY ABOVE, HYGIENA MAKES NO OTHER WARRANTY, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING. User assumes all risk and liability resulting from use of the BAX® System software, whether used singly or in combination with other products.
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, 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 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.
8. THE SOLE AND EXCLUSIVE REMEDY OF CUSTOMER/USER, AND THE SOLE AND EXCLUSIVE LIABILITY OF HYGIENA, ITS AFFILIATES, DISTRIBUTORS, LICENSORS OR REPRESENTATIVES FOR ANY AND ALL CLAIMS, INCLUDING BREACH OF WARRANTY, TORT, CONTRACT, STRICT LIABILITY, NEGLIGENCE OR OTHERWISE SHALL BE LIMITED TO THE FOLLOWING: (a) Should Equipment fail to conform with the Paragraph 2 warranty, Hygiena shall, at its option: repair or replace the non-conforming Equipment with new or refurbished (repaired or rebuilt) functionally equivalent Equipment or refund the purchase price; (b) Should BAX® System software fail to conform with the Paragraph 3 warranty, Hygiena will replace it free of charge; (c) For all other claims, Hygiena may, at its option, refund the purchase price or replace the Equipment, assays or media; (d) In all cases, user is responsible for the repackaging and return of non-conforming Equipment, along with the reinstallation of new or refurbished Equipment; and (e) Equipment, assays or media shall not be returned without prior written permission from Hygiena, and then only in the manner prescribed by Hygiena. The maximum liability of Hygiena, its affiliates, distributors and licensors, and whether or not based on negligence, shall not exceed in the aggregate the amount equal to: (a) the purchase price of the BAX® System X5 Equipment, PCR assays or media for which damages are claimed, or (b) in the case of BAX® System software, the amount paid for the software (if licensed separately) or two thousand dollars (\$2,000.00USD). Customer/user shall notify Hygiena of any claim within thirty (30) days thereof and shall commence any action against Hygiena within one (1) year of the cause of action or otherwise be barred from any remedy. Hygiena shall not be responsible for cost, loss or liabilities that arise from customer/user's operation of its business, and customer/user agrees to indemnify, defend and hold Hygiena and its representatives harmless from such cost, loss or liability.

HYGIENA™ STATMEDIA™ SOLUBLE PACKETS LIMITATION OF WARRANTY AND LIABILITY

USE OF STATMEDIA™ SOLUBLE PACKETS CONSTITUTES AN ACCEPTANCE OF ALL TERMS AND CONDITIONS OF THIS LIMITATION OF

WARRANTY AND LIABILITY. StatMedia™ soluble packets should not be used for human diagnostic or human treatment purposes or as the sole basis for assessing the safety of products for release to consumers. Use for research and development, quality assurance and quality control under supervision of technically qualified persons. Any additional or different terms in Buyer's purchase form(s) are material alterations and hereby rejected.

1. StatMedia™ soluble packets are warranted to meet standard specifications in effect on the date of shipment. User assumes all risk and liability resulting from use of the StatMedia™ soluble packets, whether used singly or in combination with other products. HYGIENA MAKES NO OTHER WARRANTY, EITHER EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING.
2. 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, EXEMPLARY, PUNITIVE 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. The sole and exclusive remedy of customer/user, and the sole and exclusive liability of Hygiena its affiliates, distributors, licensors or representatives for any and all claims, including breach of warranty, tort, contract, strict liability, negligence or otherwise shall be limited to the refund the purchase price or replacement the StatMedia™ soluble packets at Hygiena's option. The maximum liability of Hygiena, its affiliates, distributors, and licensors shall not exceed in the aggregate the amount equal to the purchase price of the StatMedia™ soluble packets for which damages are claimed. Customer/user shall notify Hygiena of any claim within thirty (30) days thereof and shall commence any action against Hygiena within one (1) year of the cause of action or otherwise be barred from any remedy

0615

BAX® System**SOFTWARE LICENSE AGREEMENT**

IMPORTANT NOTE: CAREFULLY READ THIS LICENSE BEFORE USING THE BAX® SYSTEM. YOUR INSTALLATION AND/OR USE OF THE BAX® SYSTEM INDICATES YOUR ACKNOWLEDGMENT THAT YOU HAVE READ THIS LICENSE AND AGREE TO ITS TERMS. IF YOU DO NOT AGREE, CONTACT THE SELLER FOR THE RETURN OF THE BAX® SYSTEM WITHIN 10 DAYS OF THE DATE OF PURCHASE TO ARRANGE FOR RETURN OF THE SYSTEM OR SOFTWARE, AS APPLICABLE, AND A REFUND OF THE PURCHASE PRICE LESS SHIPPING COSTS. BAX® SYSTEM SOFTWARE IS PROVIDED UNDER THE FOLLOWING LICENSE, WHICH DEFINES WHAT YOU MAY DO WITH IT AND CONTAINS LIMITATIONS ON WARRANTIES AND/OR REMEDIES. THIS LICENSE IS GRANTED BY QUALICON DIAGNOSTICS LLC, A HYGIENA™ COMPANY ("HYGIENA").

1. In return for your use of the BAX® System Software, which is protected by copyright, trade secret, and other intellectual property laws domestically and internationally, Hygiena grants to Customer a non-exclusive, non-transferable right to use one (1) copy of the BAX® System software on one (1) BAX® System or its associated CPU for the purpose of microbial detection in the fields of food/water testing and industrial microbiology only and subject to the following conditions. This license does not include the right to upgrades, updates or other enhancements to the BAX® System software, except and unless provided by Hygiena. All title and intellectual property in and to the BAX® System software and the accompanying printed materials are owned by Hygiena. This License confers no title or ownership in the BAX® System software, any associated source code or in any other intellectual property of Hygiena. Customer agrees not to disassemble, decompile, decrypt, or reverse engineer the BAX® System software except, and only to the extent, where permitted by applicable law, which permission cannot be contractually waived. BAX® System software may not be exported in violation of U.S. export restrictions. BAX® System software is delivered and licensed as "Commercial Computer Software" as defined in DFARS 252.227-7014 (June, 1995) or as or as a "commercial item" as defined in FAR 2.101(a), "Restricted Computer Software" as defined in FAR 52.227-19 (June 1987) (or any equivalent agency regulation or contract clause), as appropriate. Customer agrees that it has only those rights provided for such Software by the applicable FAR or DFARS clause or the BAX® System software terms in this Agreement. Limitations of Warranties and Liability shall continue in force even after any termination.
2. MATLAB® is deployed with the BAX® System and governed by one or more agreements between Hygiena and MathWorks. License agreement files (MathWorks or Third-Party) that are included with the MCR Libraries ("MCR Library License") may not be modified or removed.
3. **Jurisdiction:** The laws of the State of Delaware will be used to govern, construe, and enforce the rights and duties of the parties arising out of or relating in any way to the subject matter of this Agreement and the courts of Delaware shall have exclusive jurisdiction. Sale of BAX® System software shall not be governed by the U.N. convention on Contracts for the International Sale of Goods.
4. **Transfer:** This Agreement is not assignable or transferable, in whole or in part, except with the written consent of Hygiena.
5. **Limitation of Liability.** Hygiena warrants that for a period of 60 days from the date of first date of use by the Customer, BAX® System software will be free from defect in materials and workmanship and that the BAX® System software will substantially perform in accordance with the accompanying BAX® System software documentation. EXCEPT FOR THE EXPRESS WARRANTY ABOVE, HYGIENA MAKES NO OTHER REPRESENTATION OR WARRANTY, EITHER EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING OR THAT USE OF THE SOFTWARE WILL SATISFY OR ENSURE COMPLIANCE WITH ANY LEGAL OBLIGATIONS OR LAWS OR REGULATIONS.
6. CUSTOMER ASSUMES ALL RISKS IN USING THE BAX® SYSTEM (AND/OR DATA SHARING SYSTEM) AND HYGIENA OR ITS AFFILIATES, DISTRIBUTORS, LICENSORS OR REPRESENTATIVES SHALL HAVE NO LIABILITY TO CUSTOMER OR TO ANY OTHER PERSON OR ENTITY FOR ANY INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, PUNITIVE 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.
7. THE SOLE AND EXCLUSIVE REMEDY OF CUSTOMER, AND THE SOLE AND EXCLUSIVE LIABILITY OF HYGIENA, ITS AFFILIATES, DISTRIBUTORS, LICENSORS OR REPRESENTATIVES FOR ANY AND ALL CLAIMS, INCLUDING BREACH OF WARRANTY, TORT, CONTRACT, STRICT LIABILITY, NEGLIGENCE OR OTHERWISE SHALL BE LIMITED TO THE FOLLOWING: Should the software fail during the warranty period, Hygiena will replace it free of charge. The maximum liability of Hygiena, its affiliates, distributors, licensors and representatives, and whether or not based on negligence, shall not exceed in the aggregate the greater of: the amount paid by Customer for the software (if separately licensed) or two thousand dollars (\$2,000.00USD). Customer shall notify Hygiena of any claim within thirty (30) days thereof and shall commence any action against Hygiena within one (1) year of the cause of action or otherwise be barred from any remedy. Hygiena shall not be responsible for cost, loss or liability that arises from Customer's operation of its business, and Customer agrees to indemnify, defend and hold Hygiena and affiliates, distributors, licensors and representatives harmless from such cost, loss or liability.



www.hygiena.com

© 941 Avenida Acaso, Camarillo, CA 93012 | ☎ 800.863.6842 US | ☎ 302.695.5300 International