

BAX[®] System X5

User Guide

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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.

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Introduction and Overview

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 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



Hot surface! Avoid touching.



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

Ordering Information

For more information about the BAX® System and other products from Qualicon Diagnostics, contact your local representative or visit www.hygiena.com.

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 (D15407117)	BAX® System X5 Instrument Package	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 (D15407134)	BAX® System X5 Computer Package	Laptop computer pre-loaded with BAX® System X5 software Computer mouse Security cable lock
MCH2000 (D15407120)	BAX® System X5 Auxiliary Package <i>* 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.</i>	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 (D12745725)	BAX® System MP Media - StatMedia™ soluble packets	20 pouches x 5 packets per pouch x 33.75 g per packet
MED2003 (D12404925)	BAX® System MP Media - bulk powder	2.5-kg tub
MED2002 (D12232794)	BAX® System Media for <i>Listeria</i> - bulk powder	2.5-kg tub

BAX® System X5 PCR Assays		
Part Number	Description	Contents
KIT2022 (D15407214)	BAX® System X5 PCR Assay for <i>E. coli</i> O157:H7	Lysis buffer (2 x 12 mL) Protease (400 µL) PCR tubes with tablets (64) Flat optical caps for PCR tubes (96)
KIT2024 (D15407193)	BAX® System X5 PCR Assay for Genus <i>Listeria</i>	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 (D15407201)	BAX® System X5 PCR Assay for <i>L. monocytogenes</i>	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 (D15407187)	BAX® System X5 PCR Assay for <i>Salmonella</i>	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 $\pm 2^{\circ}\text{C}$
- Hypochlorite bleach (10% household solution)
- 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.
- 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

I. The BAX® System X5 Instrument

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:

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.

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.

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.

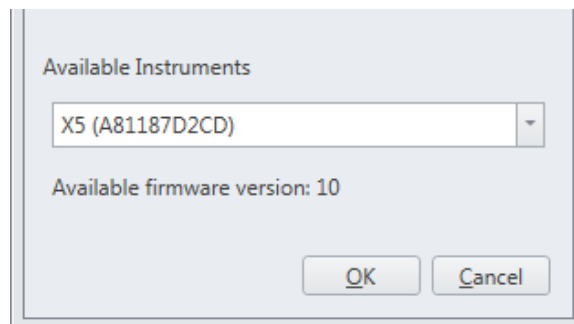
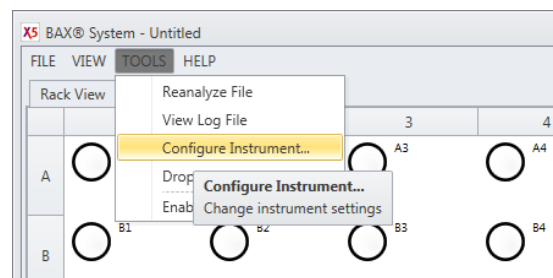
Ensure that the BAX® System X5 computer is connected to the BAX® System X5 instrument. When the instrument has a valid connection, you will see a green and yellow light above the connector.

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.)

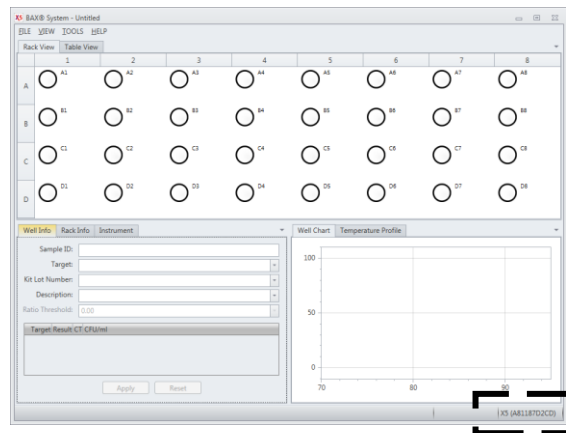
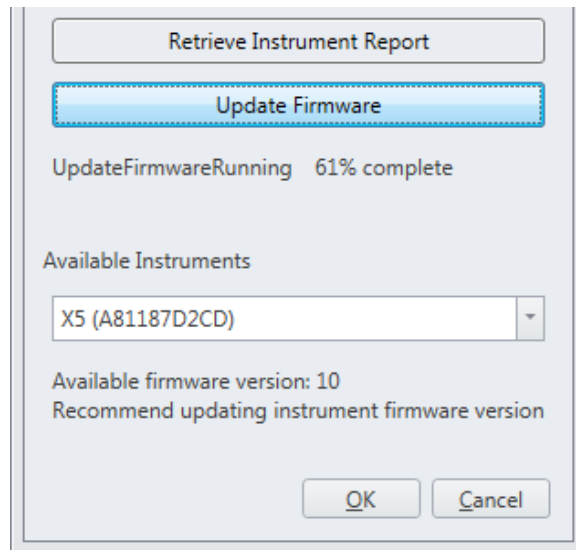
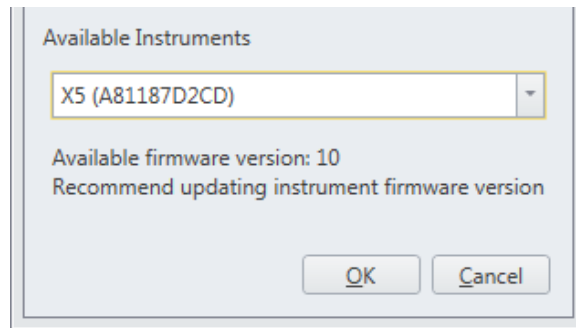
Click **OK** to confirm the selection.



*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.

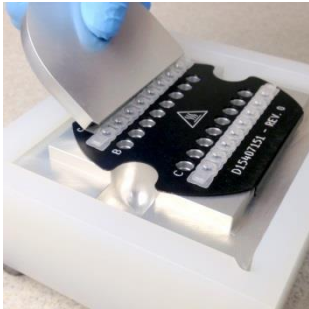
Decontaminating the Instrument Mount and Exterior Surfaces

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.

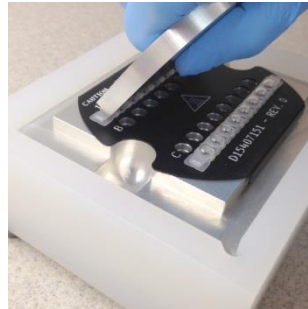
Capping/Decapping Tools

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.

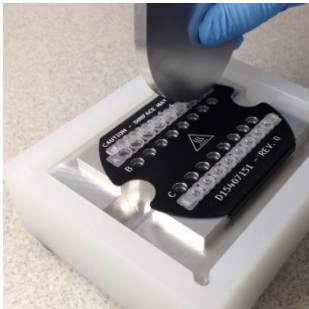


Tilt the tool forward and push down so that it locks over the caps.

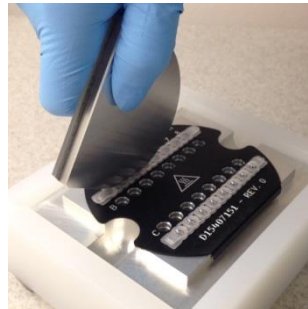


Tilt the tool backward so that the caps lift off.

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.

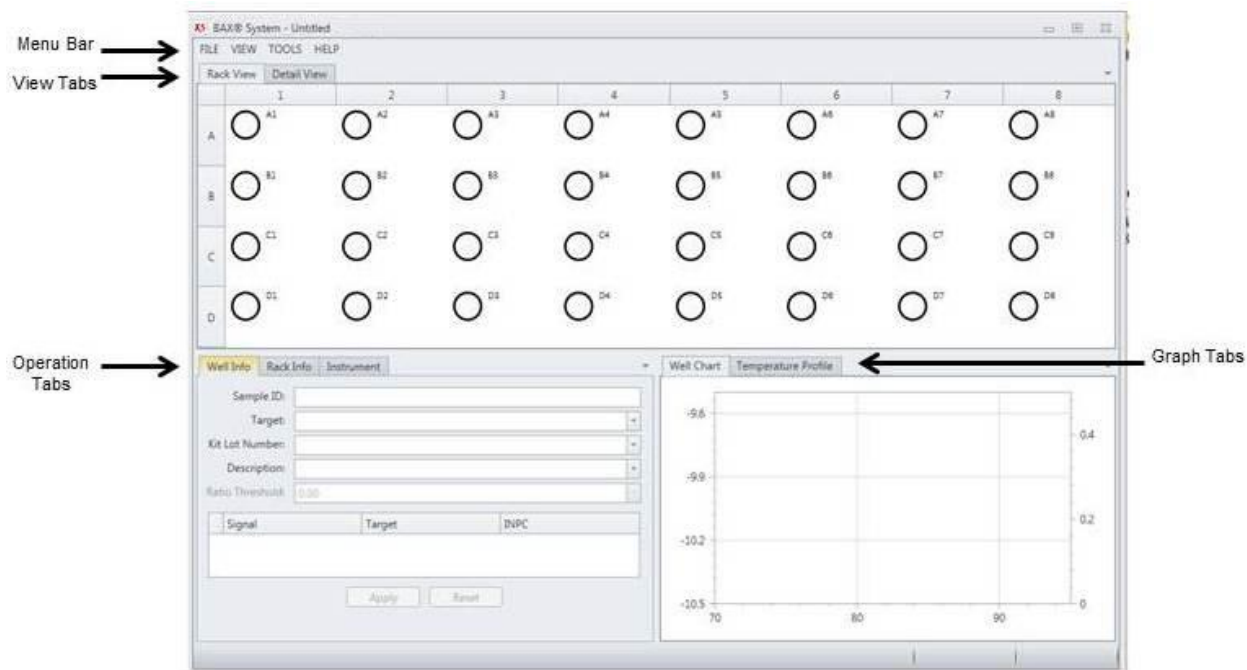
*U.S. patent 5.967.001

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

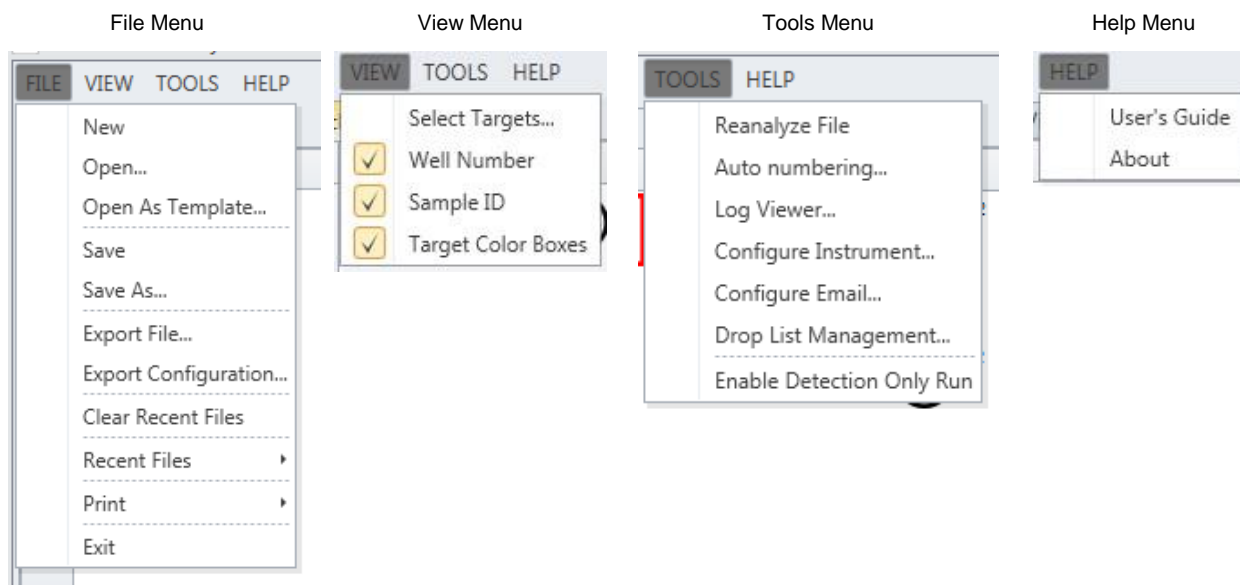
II. The BAX® System X5 Software

The BAX® System X5 Software Window

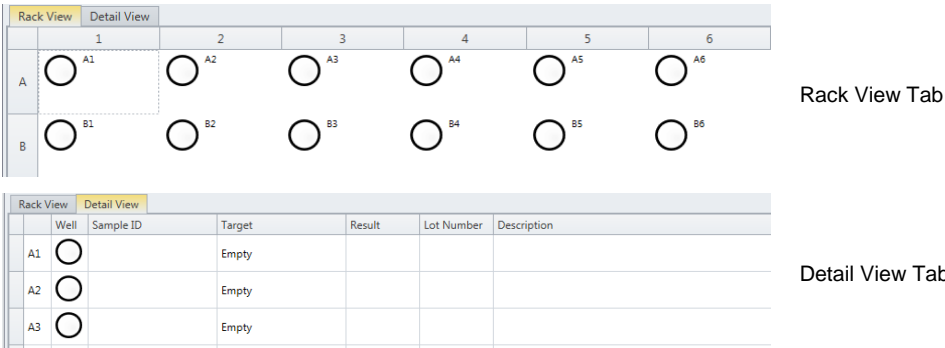
The main window is divided into these sections:



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



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



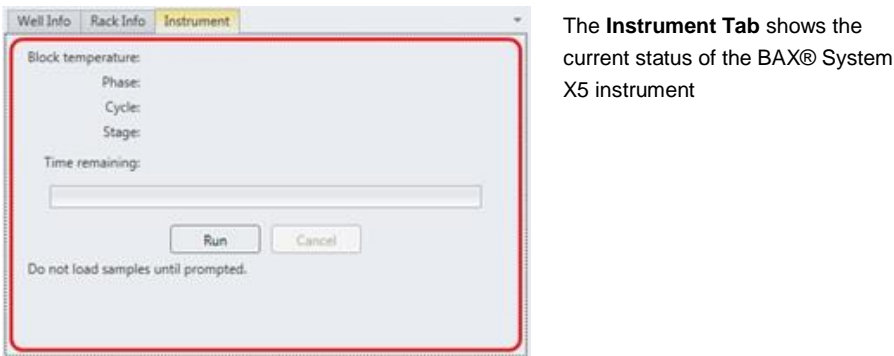
The **Operation Tabs** switch between viewing Well Info for a specific sample, Rack Info for the entire process run and Instrument status.



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

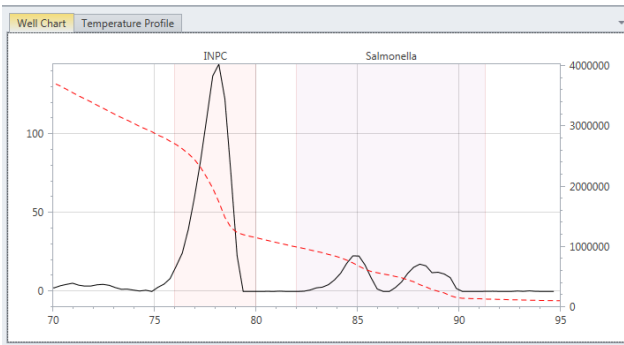


The **Rack Info Tab** allows you to enter information about all samples in the process run

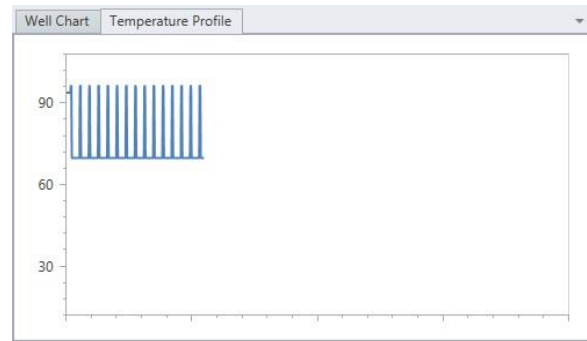


The **Instrument Tab** shows the current status of the BAX® System X5 instrument

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

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.

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

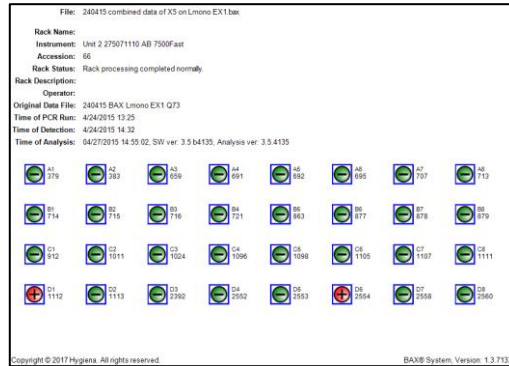
Click **APPLY** to save the entered information.

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



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

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

Rack Name: Unit 2 275071110 AB 7500Fast
 Instrument: Unit 2 275071110 AB 7500Fast
 Accession: 66
 Rack Status: Rack processing completed normally

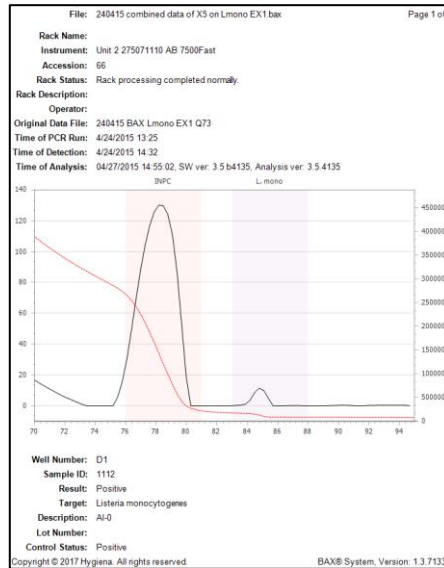
Rack Description:
 Operator:
 Original Data File: 240415 BAX Lmono EX1 Q73
 Time of PCR Run: 4/24/2015 13:25
 Time of Detection: 4/24/2015 14:32
 Time of Analysis: 04/27/2015 14:55:02, SW ver: 3.5 b4135, Analysis ver: 3.5.4135

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	2522	Negative	Listeria monocytogenes	AI-0		Positive
D5	2553	Negative	Listeria monocytogenes	AI-0		Positive
D6	2554	Positive	Listeria monocytogenes	AI-0		Positive
D7	2558	Negative	Listeria monocytogenes	AI-0		Positive
D8	2560	Negative	Listeria monocytogenes	AI-0		Positive

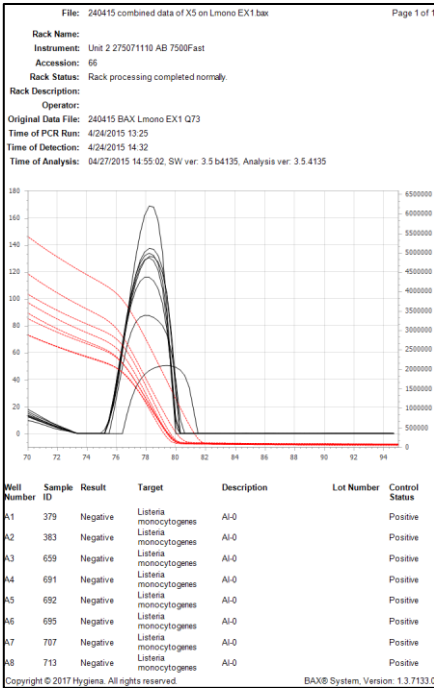
Copyright © 2017 Hygiena. All rights reserved. Hygiena™ BAX® System, Version: 1.3.7133.0

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.

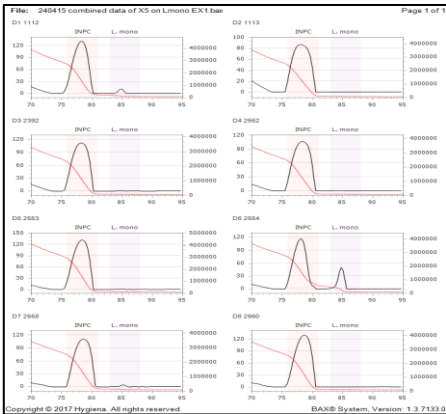
Note: To print individual well reports for multiple samples in a process run, you can highlight the desired wells in the View Tab before printing.



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.

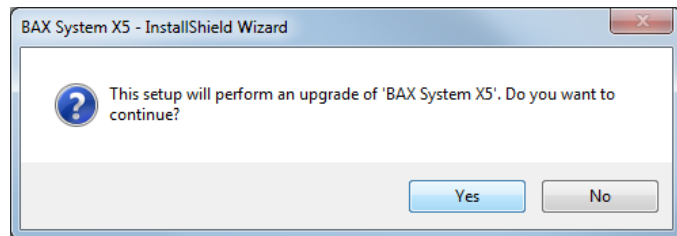
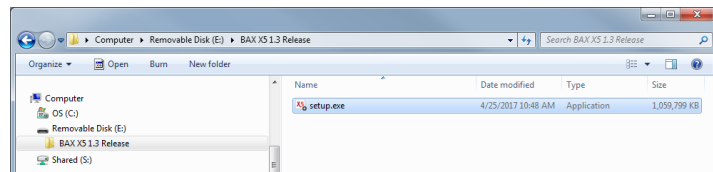
Note: The BAX® System software upgrade must be transferred to the BAX® System X5 computer, using a USB flash drive (memory stick), and network, or similar. The example below shows the process using a USB drive.

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.

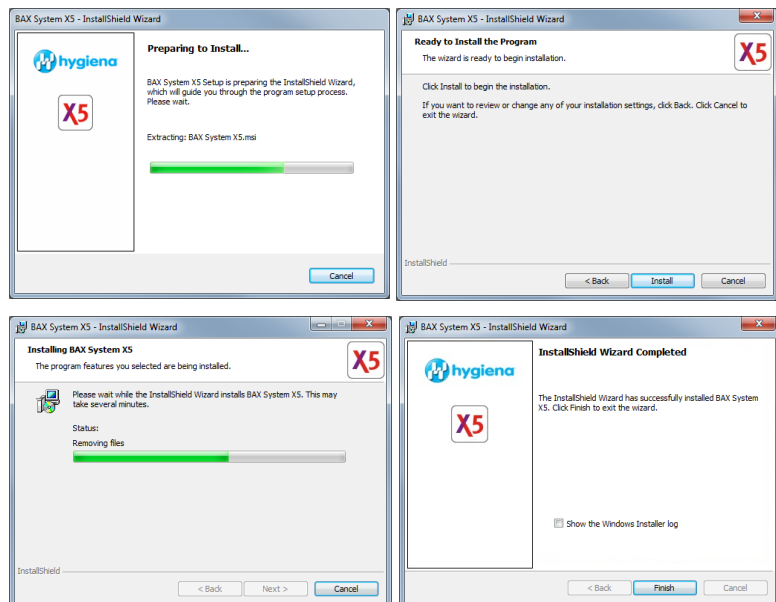
If you are doing an upgrade, a confirmation window may appear asking if you want to continue with the upgrade. Click **YES** to begin the upgrade installation.

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.



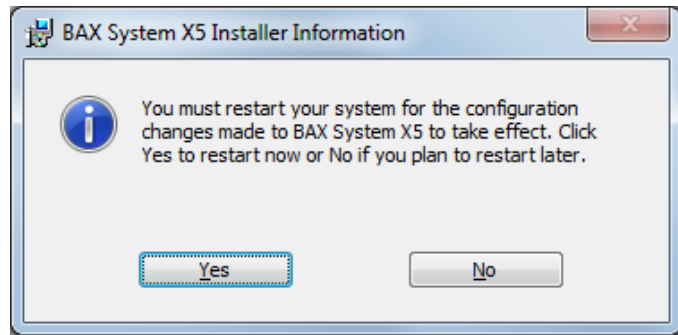
The installation wizard begins and proceeds through a number of installation screens – some examples are shown here. There may be different screens depending upon whether this is a new install or an upgrade. Click **OK** or **NEXT** to accept any modifications required for the software installation.

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

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

Kit #KIT2022 (D15407214)

1. Enrich Samples

 <p>The AOAC Research Institute has validated the BAX® System for detecting <i>E. coli</i> O157:H7 in raw ground beef, beef trim, spinach and lettuce.</p> <p>This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.</p>		
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 375 g – Gently massage 375g sample with 1.5L pre-warmed (42°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> <p>For 325 g - Gently massage 325g sample with 975mL pre-warmed (42°C) mTSB media for 30 seconds so that broth covers entire surface of sample. Incubate at 42°C for 20-24 hours.</p> <p>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>	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 375 g - Combine 375 g sample with 1.5L 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 - Combine 200 g sample with 450mL of mBPWp media and swirl to soak entire sample. Incubate at 37°C for 5 hours. After incubation 2mL of ACV supplements and incubate at 42°C for an additional 18-24 hours.</p>	<p>None</p> <p>Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.</p>

 <p>QUA 18/04 – 03/08 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS http://nf-validation.afnor.org</p>		<p>The French Association of Normalization (AFNOR Certification) has certified the BAX® System MP assay according to the NF VALIDATION rules for detecting <i>E. coli</i> O157:H7 in raw beef, raw pork, lamb, chicken, raw milk and cooked fruits and vegetables.</p>
Sample type	Enrichment	
Raw 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*	Homogenize 25 g sample with 225 mL pre-warmed (41.5°C) mTSB broth supplemented with novobiocin. Incubate at 41.5°C for 18-24 hours.	

Note: Not all enrichment protocols have not been validated by a third party organization and are subject to change during the third-party validation process. It is strongly recommended that you internally validate these enrichment protocols for your sample type before adopting the method for regular testing.

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.
6. Transfer enriched sample to the corresponding cluster tubes, using a new pipette tip for each sample.
 - a. For samples enriched in BAX® System MP media, transfer 20 µL enriched sample.
 - b. For all other samples, transfer 5 µL enriched 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

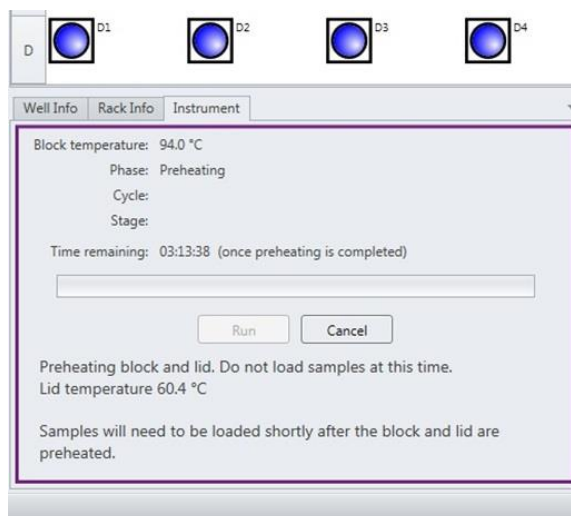
Take the rack of PCR tubes in the cooling block to the BAX® System X5 instrument.

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.

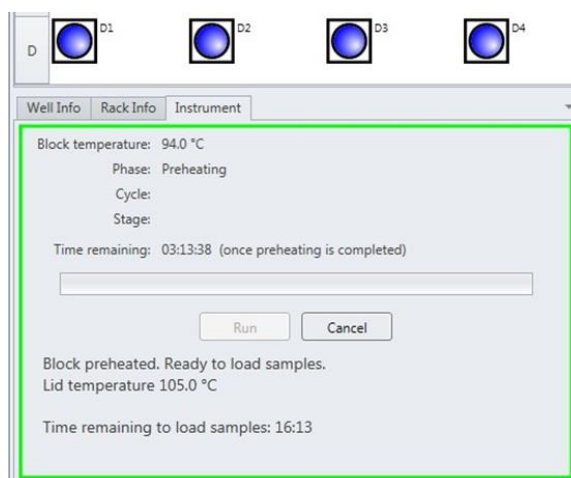


When the instrument is heated, the instrument lid LED will turn green and the instrument prompt under the status bar reads “Block preheated. Ready to load samples.”

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.

- 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.



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".

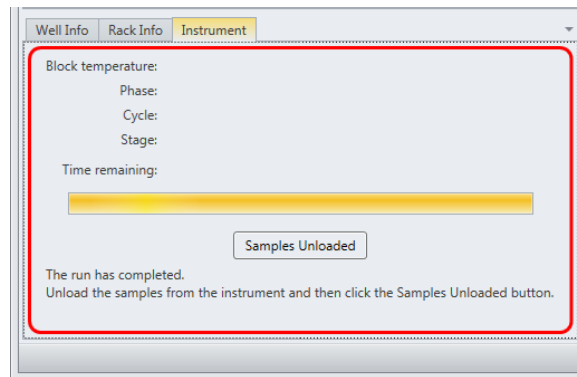
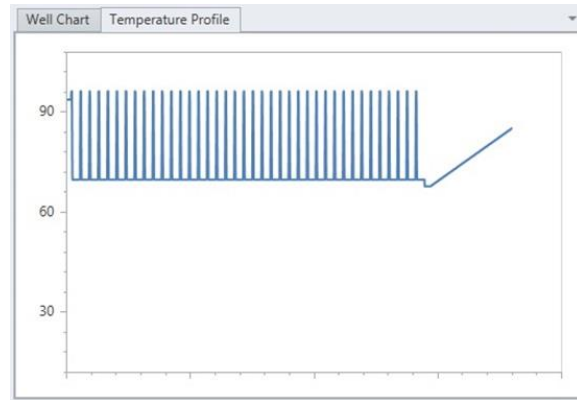
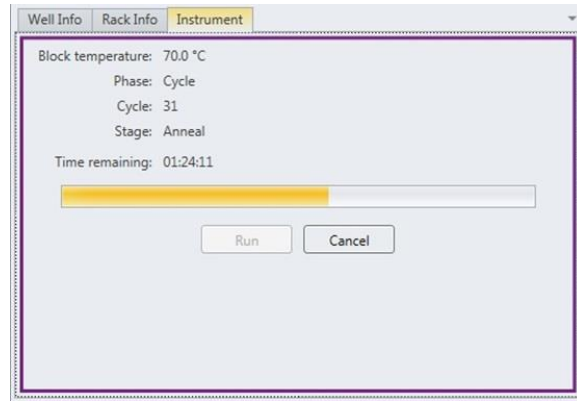
Note: Variations in the temperature profile between different process runs are normal and do not affect results.

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-cued well with an icon in the center to illustrate the result:

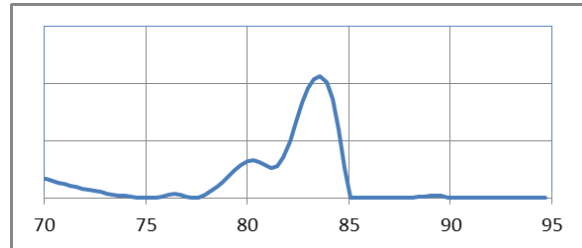
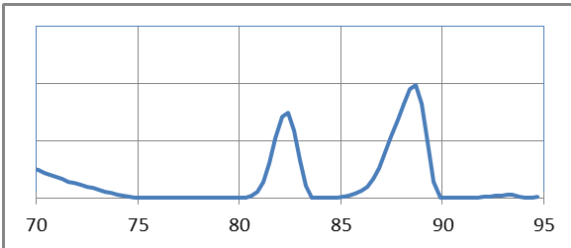
	Green (-) = Negative result		Yellow (?) = Indeterminate result
	Red (+) = Positive result		Yellow (?) with red slash = Signal error

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

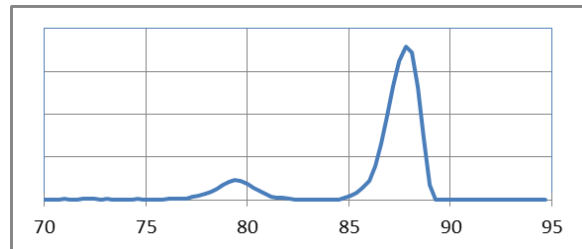
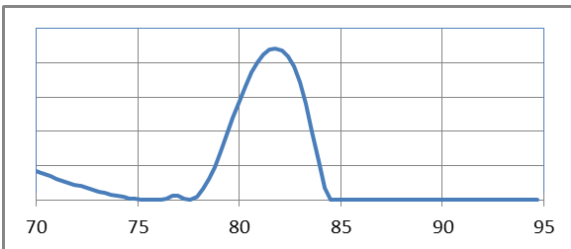


Negative *E. coli* O157:H7 MP Melting Curve Profiles

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.

Using the Genus *Listeria* Assay

Kit #KIT2024 (D15407193)

1. Enrich Samples

Sample type	Primary enrichment	Secondary enrichment
 <p>The AOAC Research Institute has validated the BAX® System for detecting Genus <i>Listeria</i> 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).</p> <p>This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.</p>		
<i>BAX® System Listeria media</i>		
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 media</i> 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 media</i> broth. Incubate at 36°C for 22-26 hours.	None
<i>Standard media</i>		
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 MOPS-BLEB. Incubate at 35°C for 18-24 hours.
Frankfurters	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. For 125 g - Homogenize 125 g samples with 1.125L of UVM media. Incubate at 30°C for 23-26 hours	Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.
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	Homogenize 25 g samples with 225 mL pre-warmed (30°C) Demi-Fraser broth. Incubate at 30°C for 22-26 hours. 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 acriflavin, nalidixic acid, and cycloheximide and incubate 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.

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 acriflavin, nalidixic acid, and cycloheximide and incubate 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.

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.

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

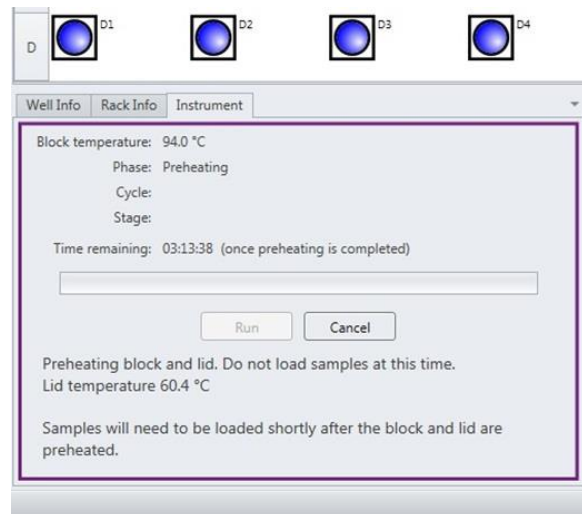
Take the rack of PCR tubes in the cooling block to the BAX® System X5 instrument.

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.

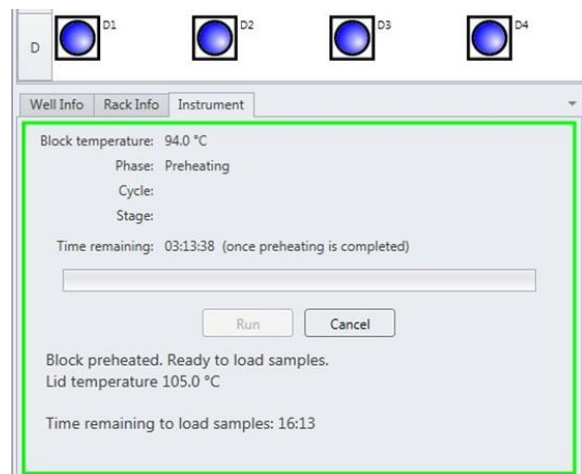


When the instrument is heated, the instrument lid LED will turn green and the instrument prompt under the status bar reads “Block preheated. Ready to load samples.”

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.

- 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.



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".

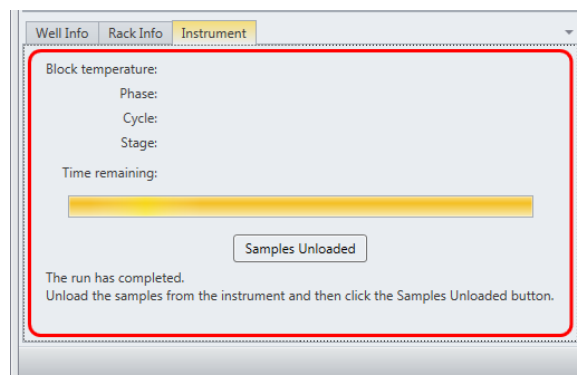
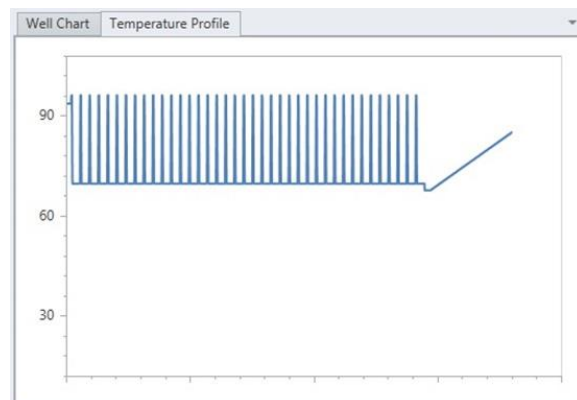
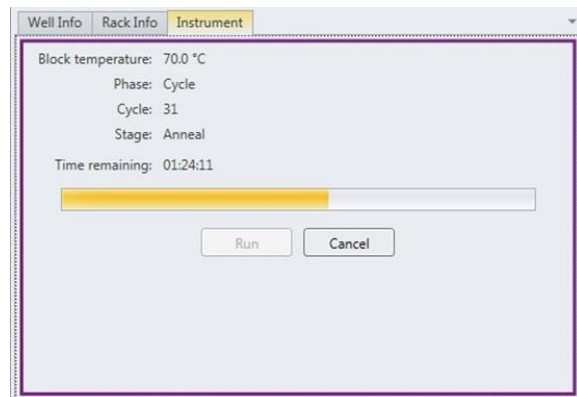
Note: Variations in the temperature profile between different process runs are normal and do not affect results.

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-cued well with an icon in the center to illustrate the result:

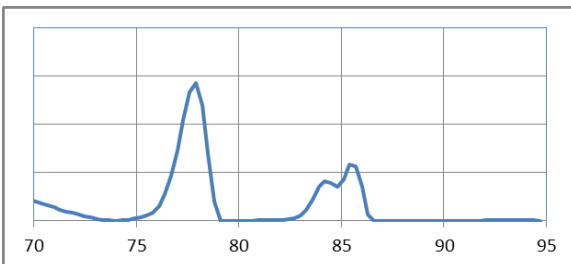
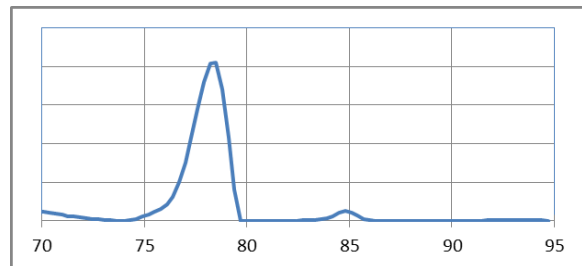
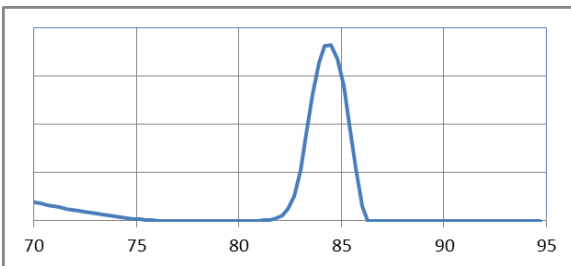
	Green (-)	=	Negative result		Yellow (?)	=	Indeterminate result
	Red (+)	=	Positive result		Yellow (?) with red slash	=	Signal error

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.

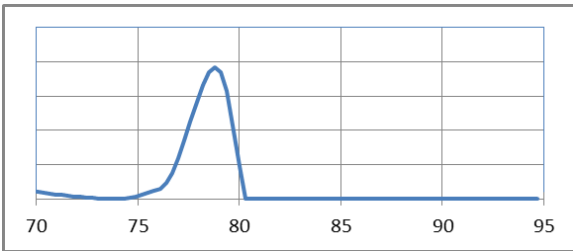
Examples of Typical Melt Curves for Positive Genus *Listeria* Samples



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 Curve for Negative Genus *Listeria* 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.

Using the *Listeria monocytogenes* Assay

Kit #KIT2023 (D15407201)

1. Enrich Samples

Sample type	Primary enrichment	Secondary enrichment
 <p>The AOAC Research Institute has validated the BAX® System for detecting <i>Listeria monocytogenes</i> in a wide variety of foods, including raw meats, fresh produce/vegetables, processed meats, seafood, dairy cultured/non-cultured, egg and egg products and fruit juices and plastic surfaces.</p> <p>This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.</p>		
Ground beef, ground pork, ground chicken, ground 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>	<p>Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.</p>
Frankfurters and pepperoni	<p>For 25 g - Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered <i>Listeria</i> Enrichment Broth 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 UVM media. Incubate at 30°C for 23-26 hours</p>	<p>Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.</p>
Fish sticks, surimi, langostinos, smoked salmon	<p>Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered <i>Listeria</i> Enrichment Broth without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours.</p>	<p>Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.</p>
Smoke Salmon	<p>Homogenize 25 g samples with 225 mL pre-warmed Universal Pre-enrichment Broth. Incubate at 35°C or 22-26 hours.</p>	<p>Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.</p>
Ice cream, milk (2%) yogurt, soft cheese	<p>Homogenize 25 g sample with 225 mL pre-warmed (30°C) Complete Selective Enrichment Broth. Incubate at 30°C for 22-26 hours.</p>	<p>Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.</p>
Cabbage slaw, peas, spinach, strawberries, apple juice and orange juice, queso fresco cheese	<p>Homogenize 25 g sample with 225 mL pre-warmed (30°C) Buffered <i>Listeria</i> Enrichment Broth without antibiotics. Incubate at 30°C for 4 hours, then add antibiotics. Incubate at 30°C for an additional 18-22 hours.</p>	<p>Transfer 100 µL enriched sample to 9.9 mL pre-warmed (35°C) MOPS-BLEB. Incubate at 35°C for 18-24 hours.</p>
Environmental sponges	<p>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) UVM. Incubate at 30°C for 20-26 hours.</p>	<p>Transfer 100 µL enriched sample to 9.9 mL pre-warmed MOPS-BLEB. Incubate at 35°C for 18-24 hours.</p>

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.

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

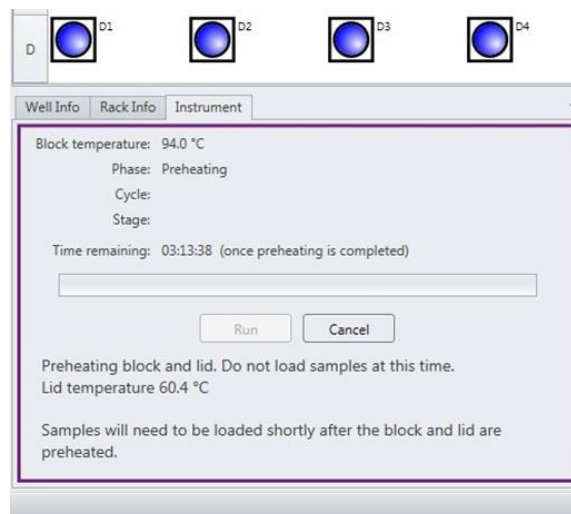
Take the rack of PCR tubes in the cooling block to the BAX® System X5 instrument.

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.

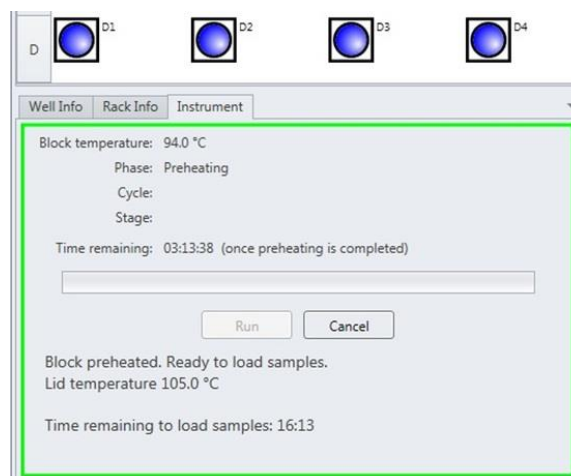


When the instrument is heated, the instrument lid LED will turn green and the instrument prompt under the status bar reads “Block preheated. Ready to load samples.”

Remove the 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.

- 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.



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".

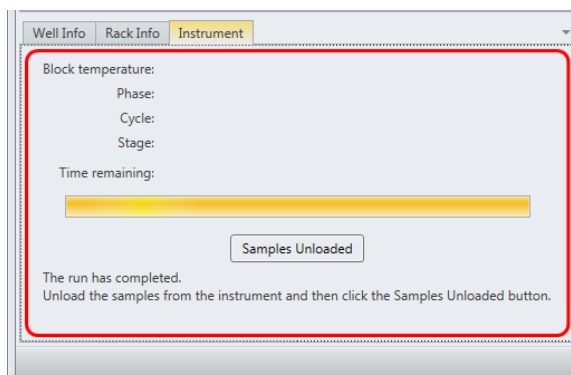
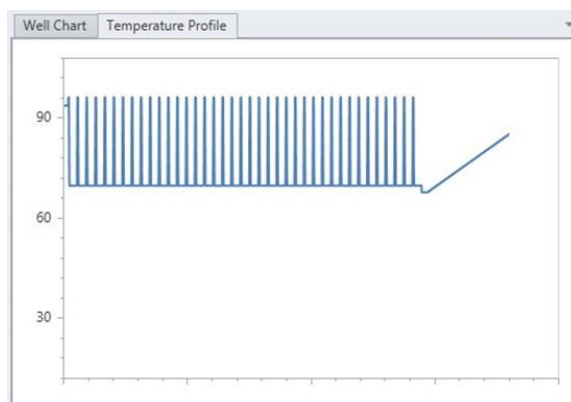
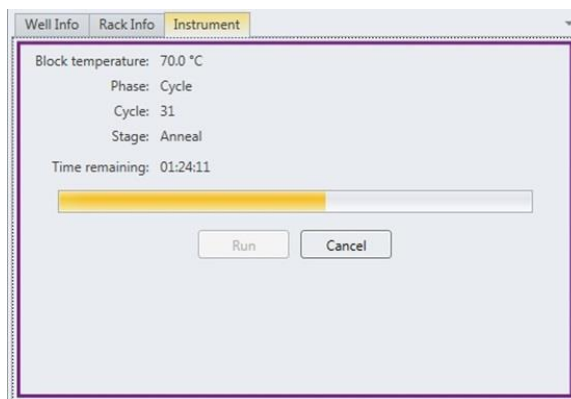
Note: Variations in the temperature profile between different process runs are normal and do not affect results.

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-cued well with an icon in the center to illustrate the result:

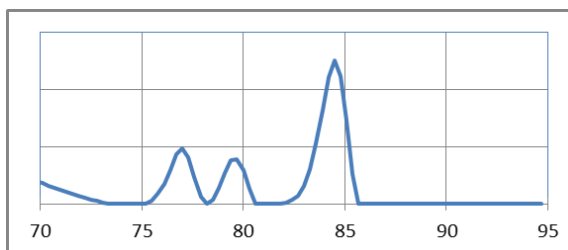
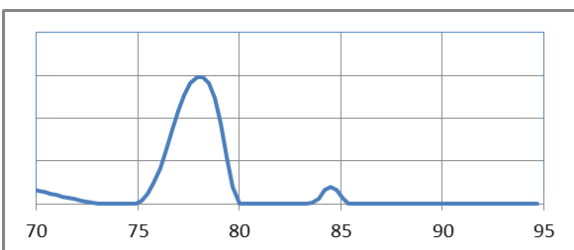
	Green (-)	=	Negative result		Yellow (?)	=	Indeterminate result
	Red (+)	=	Positive result		Yellow (?) with red slash	=	Signal error

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.

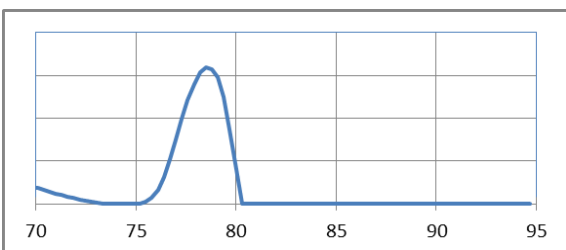
Examples of Typical Melt Curves for Positive *L. monocytogenes* Samples



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.

Using the *Salmonella* Assay

Kit #KIT2025 (D15407187)


1. Enrich Samples

Sample type	Primary enrichment	Secondary enrichment
 <p>The AOAC Research Institute has validated the BAX® System for detecting <i>Salmonella</i> in meat, poultry, fruit and vegetable products, dairy products, chocolate/bakery products, pasta, dry pet food and environmentals.</p> <p>This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.</p>		
<i>BAX® System MP media</i>		
Ground beef	<p>For 25 g - Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW media. Incubate at 35°C for 20-24 hours.</p> <p>For 25 g - Homogenize 25 g sample with 75 mL room temperature mTSB media. Incubate at 42°C for 20-24 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 9-24 hours.</p>	None
Beef trim	Gently massage 375 g sample with 1.5 L pre-warmed (45°C) BAX® System MP media. Incubate at 42°C for 9-24 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
<i>Standard media</i>		
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 with 0.45 mL 1% brilliant green dye solution per liter. 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.
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	Homogenize 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.

Custard, 2% milk, chilled ready meal, cooked fish, prawns, macaroni, pizza dough, frozen peas, orange juice, 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 LB in a flask. Let stand for 55-65 minutes at room temperature. 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.

1. Enrich Samples (continued)

Sample type	Primary enrichment	Secondary enrichment
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.
Stainless steel, ceramic tile, plastic and epoxy coated tile	Homogenize sponge with 225 mL pre-warmed (35°C) LB. Let stand for 55-65 minutes at room temperature. 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.

 <p><i>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).</i></p>		
Sample type	Primary enrichment	Secondary enrichment
<i>Standard media</i>		
Raw meats and poultry (without spices)	Homogenize 25 g sample with 225 mL room temperature BPW. Incubate at 37°C for 16-20 hours.	None
Dairy (except powdered milk)	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	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 room temperature BHI. Incubate at 37°C for 3-4 hours.

Raw beef (including seasoned and frozen) in MP media	Homogenize 25 g sample in 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.	None
Other raw meat	Homogenize 25 g sample in 225 mL pre-warmed (42°C) 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 12.5µL 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 200µL lysis reagent to each of the cluster tubes.
6. Transfer 5µL 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 50µL 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.

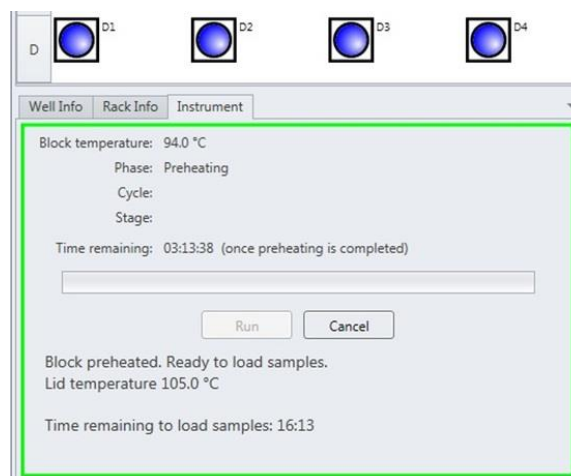
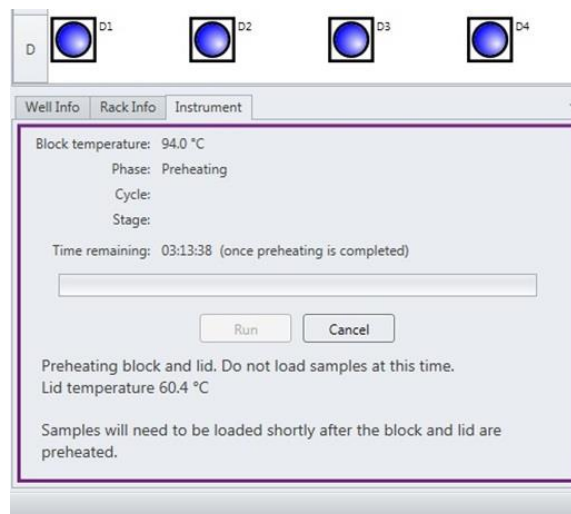
Note: Samples must be loaded within 20 minutes after the RUN button is clicked. If samples are not loaded in 20 minutes, the process run times out and you will need to re-start the process.

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 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.

- 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.



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".

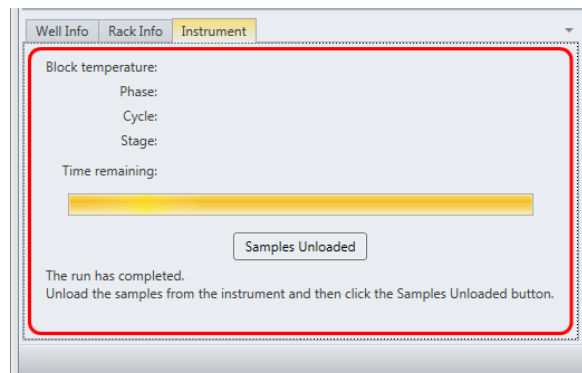
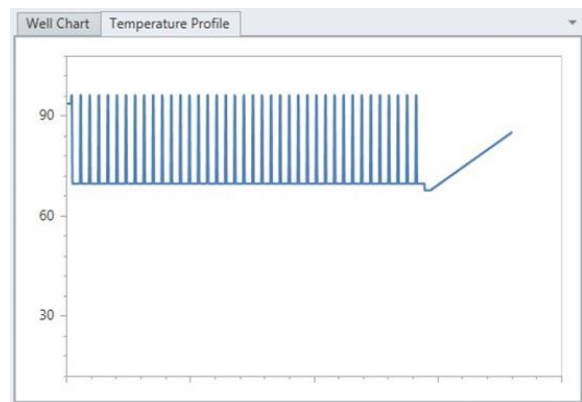
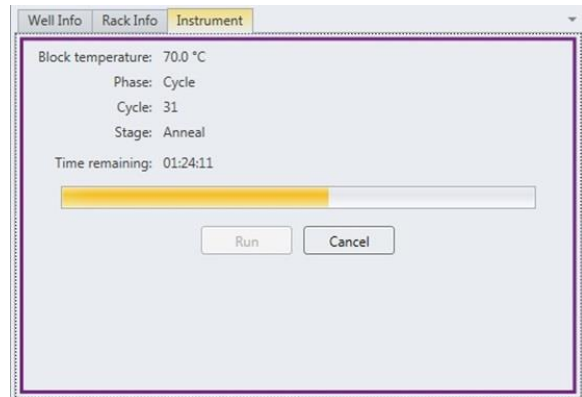
Note: Variations in the temperature profile between different process runs are normal and do not affect results.

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-cued well with an icon in the center to illustrate the result:

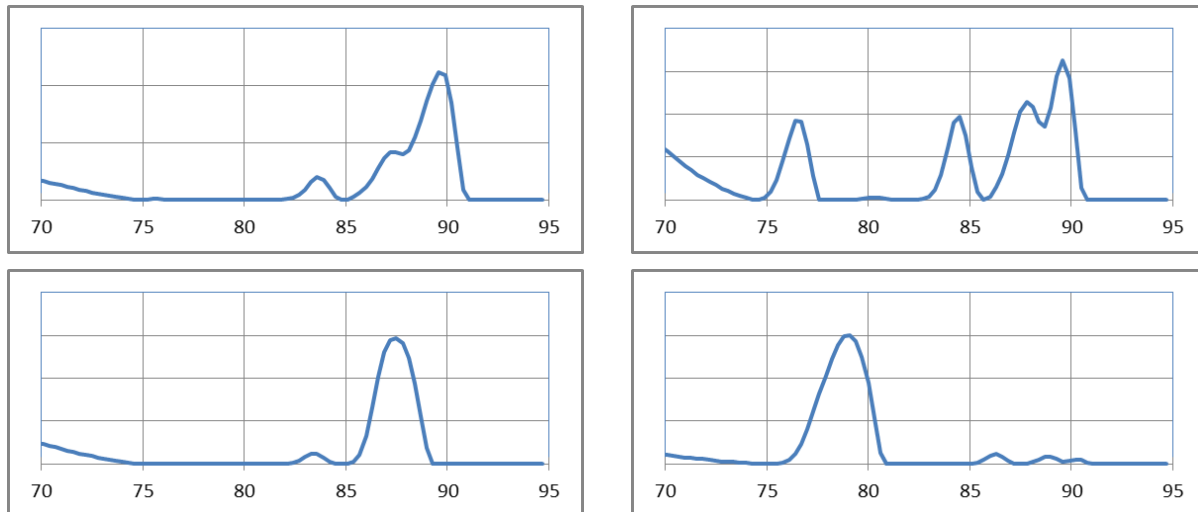
	Green (-)	=	Negative result		Yellow (?)	=	Indeterminate result
	Red (+)	=	Positive result		Yellow (?) with red slash	=	Signal error

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.

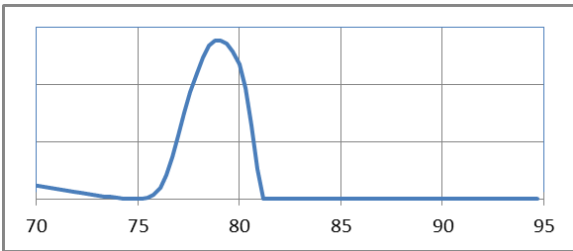
Examples of Typical Melt Curves for Positive *Salmonella* Samples



Negative *Salmonella* Melting Curve Profile

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.

Example of Typical Melt Curve for Negative *Salmonella* Sample



7. Confirm Positive Results





Method approved by AOAC

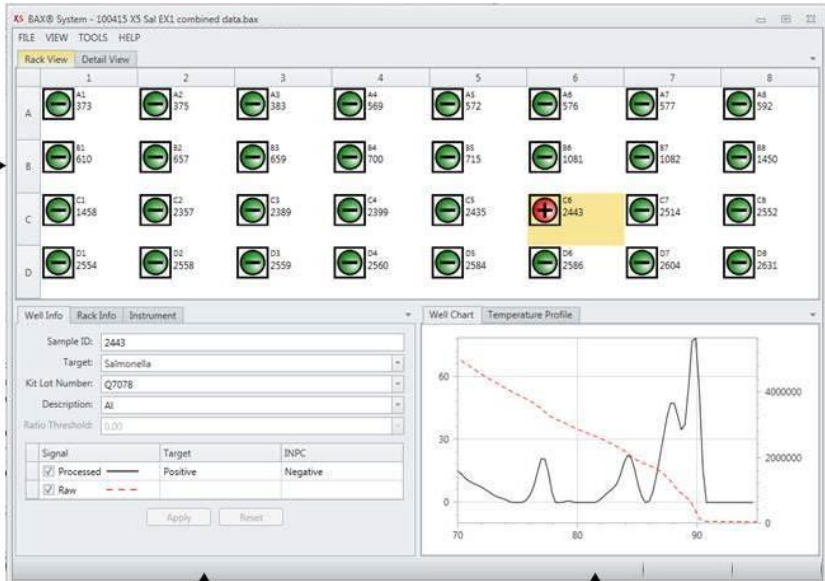
Positive results can be confirmed according to the AOAC confirmation method by following the reference culture method appropriate for the sample type. To confirm results with the FDA-BAM method, see the protocol described in the Bacteriological Analytical Manual. To confirm results with the USDA-FSIS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

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.

Results Icons

	Green (-)	=	Negative for target organism
	Red (+)	=	Positive for target organism
	Yellow (?)	=	Indeterminate result (call Diagnostics Support for assistance)
	Yellow (?) with red slash	=	Signal error (call Diagnostics Support for assistance)



The screenshot shows the BAX System software interface. At the top, there is a menu bar with 'FILE', 'VIEW', 'TOOLS', and 'HELP'. Below the menu bar is a 'Rack View' tab, which displays a grid of 32 wells (A1-D8). Each well contains a result icon and a numerical value. Well C6 is highlighted in yellow and contains a red plus sign icon and the value 2443. Below the rack view is a 'Well Info' tab, which displays detailed information for the selected sample (C6). The 'Well Info' tab includes fields for 'Sample ID: 2443', 'Target: Salmonella', 'Kit Lot Number: Q7078', and 'Description: All'. It also has a table for 'Signal' with columns for 'Target' and 'INPC', and checkboxes for 'Processed' and 'Raw'. To the right of the 'Well Info' tab is a 'Well Chart' tab, which displays a graph of the signal for the selected sample. The graph shows a signal curve with a peak at approximately 85°C, and a temperature profile curve that decreases from 90°C to 70°C. Arrows point from the text labels to the corresponding elements in the screenshot.

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

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 and Preparation

Tips for Sample Preparation

- After lysis, has been completed, unopened lysate can be stored at 2-8°C for up 7 days or at -20°C for up to 14 days before hydrating PCR tablets.
- Opened lysates may be stored for up to one week for later testing when frozen at -20°C.
- For re-testing purposes, samples can be stored after all enrichment steps have been completed, including regrowth when required. Final enrichments (after regrowth) can be stored at room temperature until test results have been reviewed and accepted (up to about 4-5 hours unless otherwise validated internally).

Note: If storing final enrichments for more than 5 hours, refrigerate at 2-8°C until test results have been accepted (up to 48 hours). Storing primary enrichments before regrowth is not recommended.

- 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.
- When preparing enrichment broth, use deionized water.

Tips for Enriching Samples

- Unless stated otherwise, all enrichment media should be pre-warmed to the specified temperature before use.
- Media should be warmed in a static incubator overnight.
- Use a thermometer to ensure incubators are within $\pm 2^{\circ}\text{C}$ of the specified temperature before placing sample in the incubator for enrichment.

Storage for Enriched and Prepared Samples

- After lysis has been completed, unopened lysate can be stored at 2-8°C for up 7 days or at -20°C for up to 14 days before hydrating PCR tablets.
- Opened lysates may be stored for up to one week for later testing when frozen at -20°C.
- For re-testing purposes, samples can be stored after all enrichment steps have been completed, including regrowth when required. Final enrichments (after regrowth) can be stored at room temperature until test results have been reviewed and accepted (up to about 4-5 hours unless otherwise validated internally).

Note: If storing final enrichments for more than 5 hours, refrigerate at 2-8°C until test results have been accepted (up to 48 hours). Storing primary enrichments before regrowth is not recommended.

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.

Tips for Transferring Samples

- Always use proper aseptic technique, including new, powder-free gloves and new filtered or barrier pipette tips for each step of sample preparation.
- Break cluster tubes apart before adding sample enrichment to help prevent cross-contamination between samples during transfer steps.

- 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.
- Do not shake or mix enrichments before transferring samples to cluster tubes. If enrichments have been agitated, let sit for at least 10 minutes before transferring aliquots to cluster tubes.
- For sample enrichments with particulates, pipette samples from the upper layer of the sample, not the bottom of the enrichment. Double-check the pipette to make sure it has retrieved the sample.
- If sample enrichments are slimy, insert only the tip of the pipette into the enrichment. 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.
- For sample enrichments with an oil/fat layer, pipette samples from the aqueous layer below the oil/fat layer. Wipe off any coating of oil/fat from the tip by scraping the tip against the side of the sample container or by using a new clean wipe or tissue for each sample. If necessary, you can transfer an aliquot from the enrichment to a secondary tube to facilitate a clean transfer to lysis tubes.
- When transferring samples from cluster tubes, draw sample from the middle of the tube so that only the liquid is drawn into the pipette. Avoid drawing sample from the bottom of the tube.

Tips for PCR Tablet Hydration

- Do not remove the PCR cooling block from the refrigerator until it is needed. Finish using the cooling block within 30 minutes of removing it from the refrigerator.
- Remove only the required number of PCR tubes from the Mylar bag. Re-seal the bag tightly and immediately refrigerate any unused PCR tubes.
- 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, complete tablet before adding sample lysate. 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 domed caps from the PCR tubes.
- Keep samples chilled at 2-8°C until immediately before loading them into the instrument.

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 heating blocks for the appropriate time as described in the table below:

BAX® System X5 Assay	Heating Step #1	Heating Step #2	Cooling Step
<i>E. coli</i> O157:H7 <i>Salmonella</i>	Place rack of cluster tubes on the heating block pre-warmed to 37°C for 20 minutes	Transfer cluster tubes to heating block pre-warmed to 95°C for 10 minutes	Place the cluster tubes into a chilled (2-8°C) cooling block for at least 5 minutes
Genus <i>Listeria</i> <i>L. monocytogenes</i>	Place rack of cluster tubes on the heating block pre-warmed to 55°C for 30 minutes		

8. Follow the instructions above to hydrate PCR tablets.
9. Follow the instructions above to complete a BAX® System process run.

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

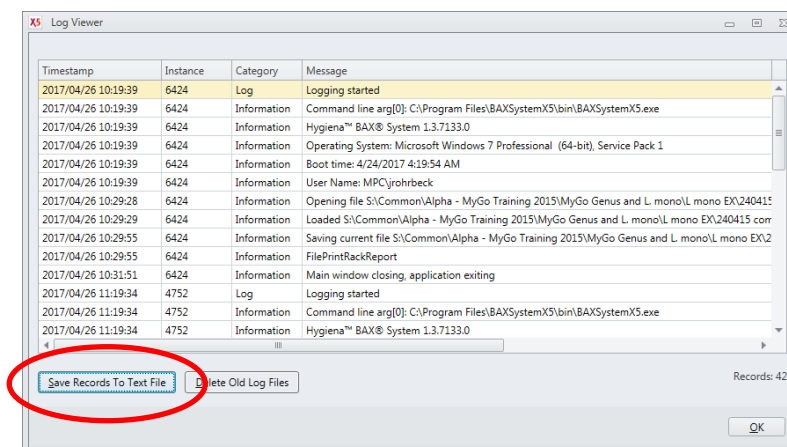
Diagnostics Support may request log files during troubleshooting. Follow these instructions to prepare the log file to send to Diagnostics Support

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

Indeterminate and error results are commonly caused by:

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

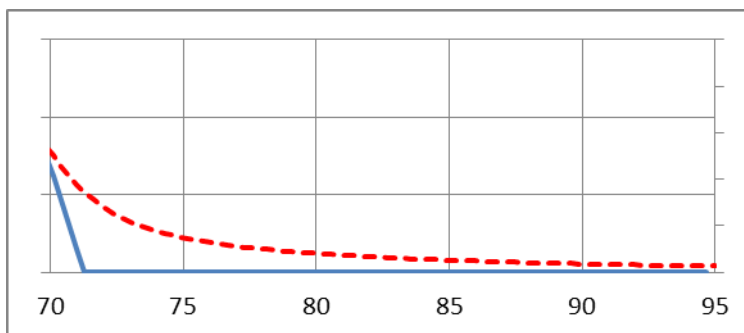
In these situations, you can examine the shape of the melt curve and its Y-axis scale to help diagnose the problem.

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.

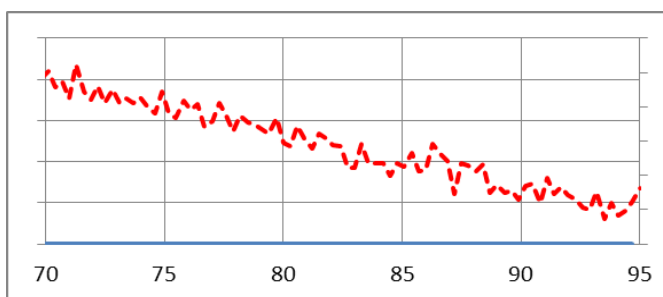


Solution - Select OPERATION > REANALYZE WELLS and follow the screen prompts. The associated data will display as captured.

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.



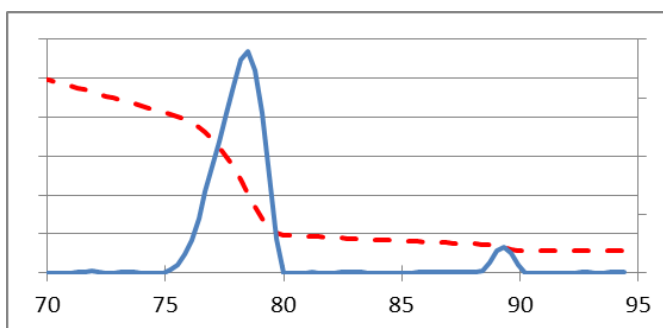
Solution – Re-label the wells in your file according to the sample placement in the rack and assign the appropriate target. Select OPERATION > REANALYZE WELLS and follow the screen prompts. The associated data will display as captured.

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.

Causes – 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.



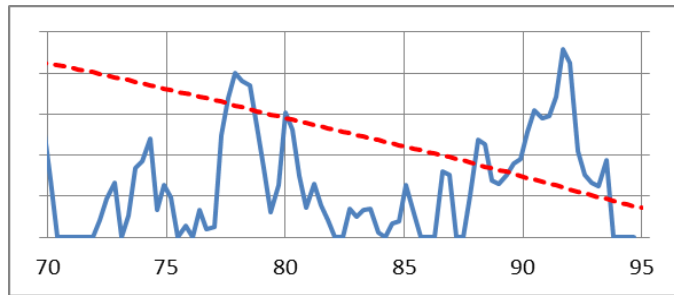
Solution - Repeat the assay from last enrichment. Follow all steps of the protocol exactly as described in this User Guide or the assay's package insert.

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

Causes – 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



Solution - 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.

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.

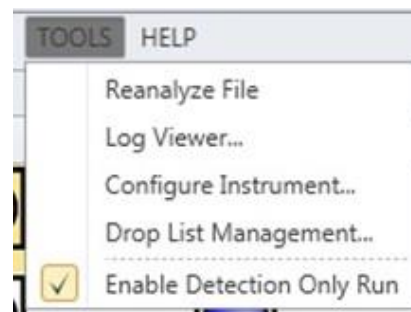
Do not use the Detection Only process unless instructed to do so by Hygiene Diagnostics Support or your local Hygiene representative or distributor.

*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.



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".

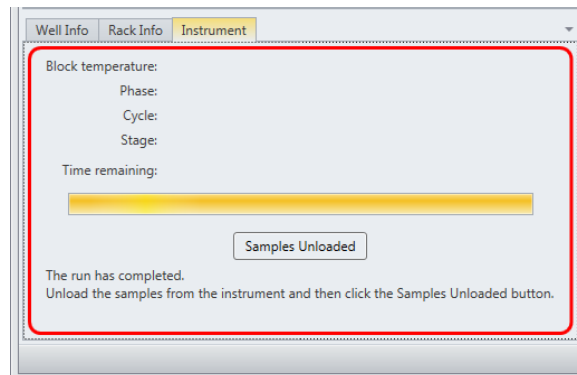
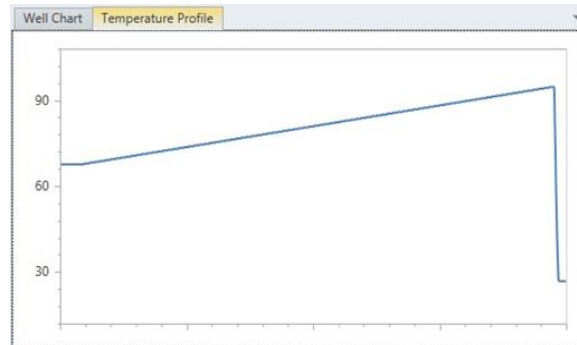
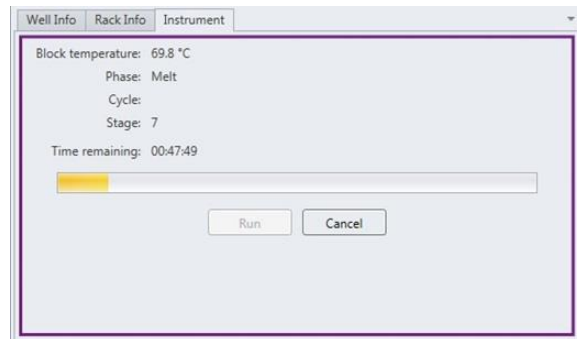
Note: Variations in the temperature profile between different process runs are normal and do not affect results.

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.



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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.
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