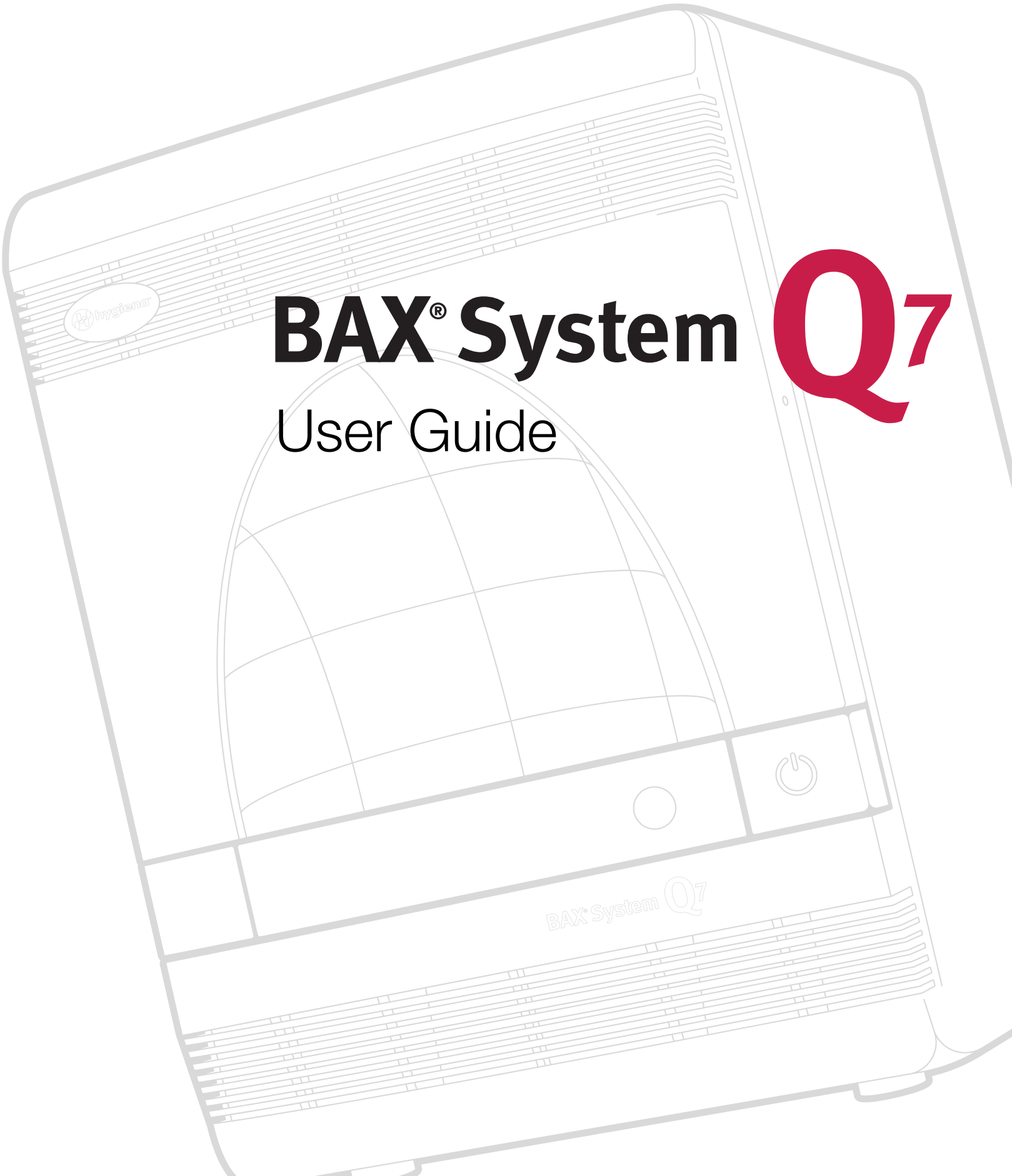
hygiena®

BAX® System Q7

User Guide



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I. User Information and Terminology

Preface

The BAX System and this *User Guide* have been designed for use in quality control/assurance laboratories of food processing and related industries.

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 II, but the main categories are as follows:

General alert or warning

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

Hot surface! Avoid touching.

II. BAX System Method Overview

About the BAX® System

The BAX System is an automated molecular method for detecting microbes in food and environmental samples. The BAX System combines speed and ease of use with unprecedented performance to give you fast, accurate and reliable results.

Traditional screening methods are based on bacterial traits or behavior, such as antibody response, which can present problems with cross-reactivity of related organisms. The BAX System, however, focuses on the actual genetic structure of bacteria by detecting a unique DNA fragment found only in the target organism.

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. Thus, you get clearly detectable "yes-or-no" answers within hours of starting the assay, without the need for expert interpretation. The BAX System Q7 series provides additional capabilities for more information with selected tests, such as faster processing time, multi-target analysis in the same tube, species differentiation and quantitative results.

The BAX System simplifies PCR in your lab. All necessary reaction reagents (primers, polymerase, nucleotides and positive control) are already combined into a single tablet, conveniently packaged inside the PCR tubes you receive with each assay. This eliminates the multiple liquid transfers used in other methods and effectively reduces the potential for errors caused by operator technique. The proprietary tablets also allow for efficient processing of large numbers of samples, up to 96 tests in a single batch.

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

This *User Guide* describes how to use the BAX System when screening food or environmental samples for targeted organisms.

About the Polymerase Chain Reaction (PCR)

PCR is an analytical tool for quickly replicating a targeted DNA fragment. 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 undergoes 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 DNA sequence. Taq polymerase then uses the 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 End-Point Detection

The BAX System PCR tablets in standard assays contain fluorescent dye, which binds with double-stranded DNA and emits a fluorescent signal in response to light. 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 can be plotted against temperature to generate a melting curve, which is interpreted by the BAX System software as positive or negative results.

About Real-Time Detection

The BAX System PCR tablets used in real-time assays contain multiple target-specific, dye labeled probes. Probes are short oligonucleotides with quencher dye at one end that greatly reduces fluorescence from the fluorophore dye at the opposite end. During PCR, probes bind to a specific area within the targeted fragment and the fluorophore is separated from the quencher, allowing for increased fluorescent signal. The BAX System Q7 instrument uses dye-specific filters to measure signal at the end of each cycle and report positive/negative results for each target.

About the BAX System Q7 Instrument

Your purchase of the BAX System includes the Q7 instrument with a computer workstation operating on a Microsoft Windows® platform. The workstation includes removable media drives, monitor, keyboard, mouse and cables. The BAX System application is already loaded and ready for use.

Note: *Although the instrument and its peripherals can be connected to a network, Hygiena cannot provide technical support for problems that arise from using this workstation on a network. Furthermore, Hygiena cannot warrant BAX System results if this computer is used with other, potentially incompatible, software.*

BAX System Q7 hardware

Item	Specification
Dimensions	14" wide x 18" deep x 20" high (34 x 45 x 49 cm)
Weight	Approx. 75 pounds (34.1 kg)
Power usage	950 watts
Nominal current draw	8 A
Power requirements	100-240 VAC ± 10%, 50-60 Hz ± 1%, 15 amp circuit, grounded outlet
Fuses	Two 12.5 A, 250 V, 5 x 20 mm
Filters	5 excitation, 5 emission
Halogen bulb	12 V, 75 W
Thermal range	4 to 100 °C
Thermal homogeneity	± 1.0 °C well-to-well within 30 seconds of reaching 60 °C
Thermal accuracy	± 0.5 °C (35 °C to 95 °C) 3 minutes after reaching set point temperature
Ramp speed	± 1.6 to 3.5 °C per second
Warm up time	5 minutes or less from 25 °C start
Sound	65 decibels maximum
Room environment	Altitude at or below 2000 m (6500 ft) above sea level Temperature 10 to 35 °C (50 to 95 °F) Relative humidity 20-80% (non-condensing) Locate away from heaters, cooling ducts, and out of direct sunlight. Protect from accidental spills. Non-conductive pollutants only
Sample throughput	1 to 96 tests per batch, time dependent on assay
Delivered with system	Computer workstation with USB drive, monitor, keyboard, mouse and cables Microsoft® Windows® operating system and BAX® System application BAX System documentation package

Symbols and Labels

Electrical Symbols

Symbol	Description
	Indicates the On/Off position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Safety Labels

English	Français
CAUTION Hazardous chemicals. Read the Safety Data Sheets (SDS) before handling.	ATTENTION Produits chimiques dangeureux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	ATTENTION Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to SDS and local regulations for handling and disposal.	ATTENTION Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	AVERTISSEMENT Lampe brûlante
WARNING This instrument is designed for 12V, 75W Halogen lamps only.	AVERTISSEMENT Cet instrument est concu pour des lampes d'halogéne de 12V et 75W seulement.
CAUTION Hot surface.	ATTENTION Surface brûlante.
DANGER High voltage.	DANGER Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Hygiena qualified service personnel.	AVERTISSEMENT Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l ' utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Hygiena.
CAUTION Moving parts.	ATTENTION Parties mobiles.

Safety Considerations

General Instrument Safety

Moving and lifting the instrument	**CAUTION - PHYSICAL INJURY HAZARD.** If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.
Moving and lifting stand-alone computers	**WARNING** - Do not attempt to lift or move the computer without the assistance of others. Depending on the weight of the computer, moving it may require two or more people. Things to consider before lifting the computer: • Make sure that you have a secure, comfortable grip on the computer when lifting. • Make sure that the path from where the object is to where it is being moved is clear of obstructions. • Do not lift an object and twist your torso at the same time. • Keep your spine in a good neutral position while lifting with your legs. • Participants should coordinate lift and move intentions with each other before lifting and carrying. • Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.
Operating the instrument	Ensure that anyone who operates the instrument has: • Received instructions in both general safety practices for laboratories and specific safety practices for the instrument. • Read and understood all applicable Safety Data Sheets (SDS). **WARNING - PHYSICAL INJURY HAZARD**. Use this instrument as specified by Hygiena to avoid personal injury or damage to the instrument

Electrical Safety

Moving and lifting the instrument	 **DANGER - ELECTRICAL SHOCK HAZARD**. Severe electrical shock can result from operating the BAX System Q7 without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.
Fuses	 **DANGER - FIRE HAZARD**. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.  **WARNING - FIRE HAZARD**. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.
Power	 **DANGER - ELECTRICAL HAZARD**. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.  **DANGER - ELECTRICAL HAZARD**. Use properly configured and approved line cords for the voltage supply in your facility.  **DANGER - ELECTRICAL HAZARD**. Plug the system into a properly grounded receptacle with adequate current capacity
Over-voltage rating	The BAX System Q7 has an installation (over-voltage) category of II and is classified as portable equipment.

Physical Hazard Safety

Moving parts	 **WARNING - PHYSICAL INJURY HAZARD**. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.  **DANGER - PHYSICAL INJURY HAZARD**. Do not operate the instrument with its door open. Keep hands out of the sample block area when the instrument is running.
Lamp	 **WARNING - PHYSICAL INJURY HAZARD**. The lamp can become very hot while in use. Allow sufficient time for the lamp to cool, and put on protective gloves before handling it.
Ultraviolet Light	 **WARNING - ULTRAVIOLET LIGHT HAZARD**. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing

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."
c UL US	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 CE	**Safety** 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." **EMC** 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."
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."

Required Materials

BAX System Kits	Contents
Standard and Real-Time PCR Assays (96-test kits)* *STEC Panel 1 and 2 assays are sold as 48-test kits. **_Cronobacter_ and Yeast & Mold assays use their own ES Lysis Buffer.	• Lysis buffer (2 x 12 mL) • Protease (400 µL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96)
Real-Time PCR Assays for Genus *Listeria* and *L. monocytogenes* (96-test kits)	• Lysis buffer (2 x 12 mL) • Protease (400 µL) • Lysing agent 2 (1.1 mL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96)
24E PCR Assays for Genus *Listeria* and *L. monocytogenes* (96-test kits)	• Lysis buffer (2 x 12 mL) • Protease (400 µL) • Lysing agent 1 (3 mL) • Lysing agent 2 (1.1 mL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96)
PCR Assay for Yeast and Mold (96-test kit and supplement kit)	• YM Lysis buffer (2 x 12 mL) • Protease (400 µL) • PCR tubes with tablets (96) • Flat optical caps for PCR tubes (96) **Supplement Kit:** • Yeast and Mold disrupter tubes (96) • DNA stabilizer (2 x 1 mL)
BAX System Media	**Contents**
BAX System MP Media	Available in 2.5-kg tubs (MED2003), 10 kg tubs (MED2029) or StatMedia™ soluble packets (33.75g) (MED2016)
BAX System Media for *Listeria*	Available in 2.5-kg tubs (MED2002)
24 LEB enrichment broth for *Listeria* assays	24 LEB Complete - available in 2.5-kg tubs (Hygiena MED2005 or Oxoid CM1154) Buffer supplement - available in packages of 20 tubes each (Hygiena MED2000 or Oxoid BO1204)
Buffered Peptone Water	Buffered Peptone Water - available in 2.5-kg (MED2010) and 500 gram tubs (MED2011)

Required Materials (Continued)

BAX System Equipment/Supplies	Contents
Start-Up Package (equipment and supplies for 192 tests)	**Equipment:** • BAX System Q7 instrument • Computer workstation • Heating blocks with inserts capable of maintaining temperatures within ± 2 °C or ± 3 °C (2)* • Cooling blocks with inserts (3)* • Capping/decapping tools (2) • Adjustable mechanical pipettes (2) • Repeating pipette (1) • Multi-channel pipette (1) **Supplies:** • Cluster tubes with caps and racks • Pipette tips with barriers (100 µL, 250 µL, 5.0 mL) • Powder-free nitrile gloves
Additional equipment and supplies – customer-supplied	• Homogenizer to mix samples between 200-260 rpm (or equivalent) • Filtered homogenizer bags (unless otherwise specified) • Incubators capable of maintaining temperatures within ± 2 °C** • Cell disrupter device (2.0 mL) for yeast and mold • Hypochlorite bleach (10% household solution) • Standard solutions, consumables and media *Uninterruptible power source for BAX System instrument and/or computer – 1980 Watts / 2200 VA output (optional)* *Line noise filter - 2.2 kVa (optional)* *Surge protector – 10 kVa (optional)* • Refrigerator for storing kits (ability to hold temperature from 2 to 8 °C) • Laboratory balance for weighing media and samples - 5kg capacity • pH measurement device for measuring media pH

** The BAX System Start-Up Package is available with either separate heating and cooling blocks or the Automated Thermal Block.*
*** Health Canada and AFNOR Certifications standards require an incubator capable of maintaining ± 1 °C*

A Note on Heating Block Temperature Stability

The heating blocks provided with the BAX System Start-Up Package demonstrate a temperature stability of ± 2 °C across the block when set at 37-55 °C and a stability of ± 3 °C across the block when set at 95 °C. These heating blocks have been used reliably in both internal and third-party validation studies and are approved by Hygiena and third-party organizations such as AOAC as part of the BAX System method.

A Note on Heating Block Temperature Stability (Continued)

These temperature variances mean that when the temperature of the heating block is set to 37 °C (or 55 °C) according to the BAX System method, the actual temperature of a single well in the heating block may read between 35-39 °C (or 53-57 °C) without affecting the method performance.

When the temperature of the heating block is set to 95 °C, the actual temperature of a single well in the heating block may read between 92-98 °C without affecting the method performance.

Before using any heating blocks with the BAX System method, verify that the blocks are capable of maintaining a temperature stability of ± 2 °C when set at 37-55 °C and a stability of ± 3 °C when set at 95 °C.

Materials Handling, Storage and Disposal

Precautions

Cycler/Detector

The instrument requires a constant supply of air that is 31 °C or cooler in order to remove heat generated by operation. If the air supply is inadequate or too hot, the machine can overheat, causing performance problems, software error messages, and even automatic shutdowns. Please see the guidelines for installation in Chapter V: BAX System Hardware.

The cycler/detector can generate enough heat to inflict serious burns and can deliver strong electrical shocks if not used according to the directions in this manual. Please read the safety considerations in Chapter II: BAX System Method Overview before using this instrument for the first time.

Reagents and Supplies

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 Hygiena's website.
Refer to your site practices for safe handling of materials at extreme temperatures.
www.hygiena.com

Storage

Reagents should be used by the expiration date printed on the individual labels.
Reagent packages should be kept 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 bag of PCR tubes into a larger bag with desiccant or store at 4 °C in a desiccation unit, if possible.

Note: *Storage of PCR tubes with desiccant is particularly important for real-time assays.*

After protease has been added to the lysis buffer, shelf life of the solution is two weeks when stored at 2 to 8 °C.

Diluted Lysing Agent 1, used with the 24E assays, can be stored up to 6 months at 20-30 °C.

After combining diluted Lysing agent 1 and Lysing agent 2, the mixture must be used within 4 hours.

After protease and Lysing Agent 2, used with the real-time *Listeria* assays, have been added to the lysis buffer, shelf life of the solution is one week when stored at 2 to 8 °C.

Cooling blocks should be kept refrigerated at 2 to 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.

Please see the manufacturer's documentation for handling, disposal and storage of the pipettes, computer system and other equipment.

Disposal

Decontaminate materials and dispose of biohazardous waste according to your site practices and as required by federal, state and local regulations.

For additional recommendations about preventing, identifying and removing PCR contamination, see Appendix B: PCR Contamination Control.

Technical Assistance

If you have any questions or comments on the BAX System, contact Hygiena Diagnostics Support directly by phone at +1 800-863-6842 or by email at techsupport@hygiena.com.

Assay Validation

The PCR protocols have been validated by Hygiena and certified by organizations such as AOAC, AFNOR, Health Canada or NordVal, for the sample types listed. Many food types use standard enrichment protocols. However, certain food types have been validated using specific media or enrichment protocol modifications.

In order to follow the methods approved by AOAC or AFNOR Certification, the validated enrichment protocols described in the kit instructions must be followed.

For many sample types, reference 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.

The reference method enrichment protocols described reflect those in use at the time of the assay's validation. Because these may have changed since that time, you should always refer to the appropriate reference method source for the most current enrichment protocol.

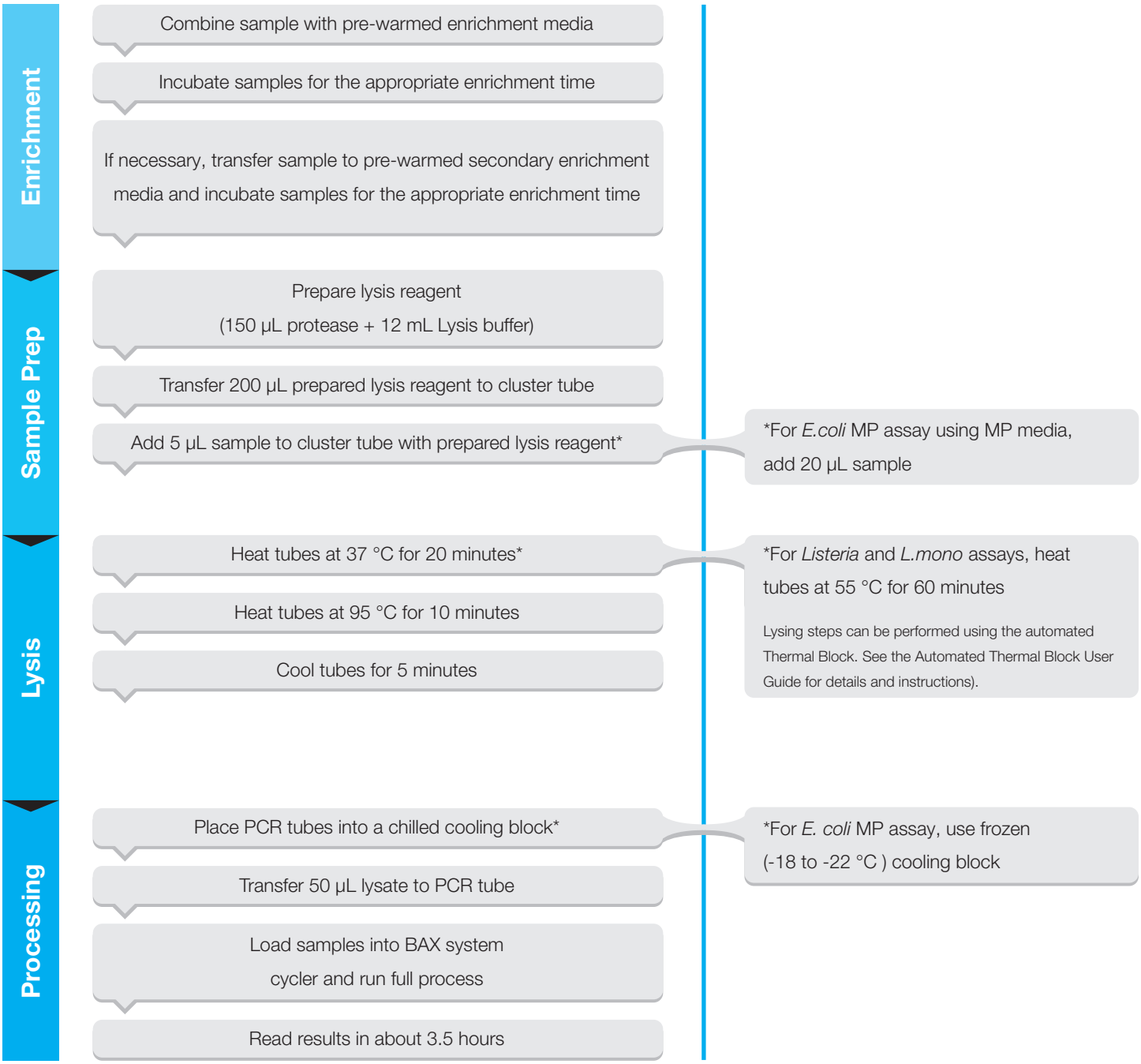
Reference Method Source
USDA-FSIS Microbiology Laboratory Guidebook (MLG)
FDA Bacteriological Analytical Manual (BAM)
Health Canada Compendium of Analytical Methods
AOAC (Association of Official Analytical Chemists) International
International Organization for Standardization (ISO)

III. BAX System Protocol Overviews

Before You Begin

Review all best practices, tips and techniques described in Chapter IV: BAX System Best Practices before beginning any BAX System protocol.

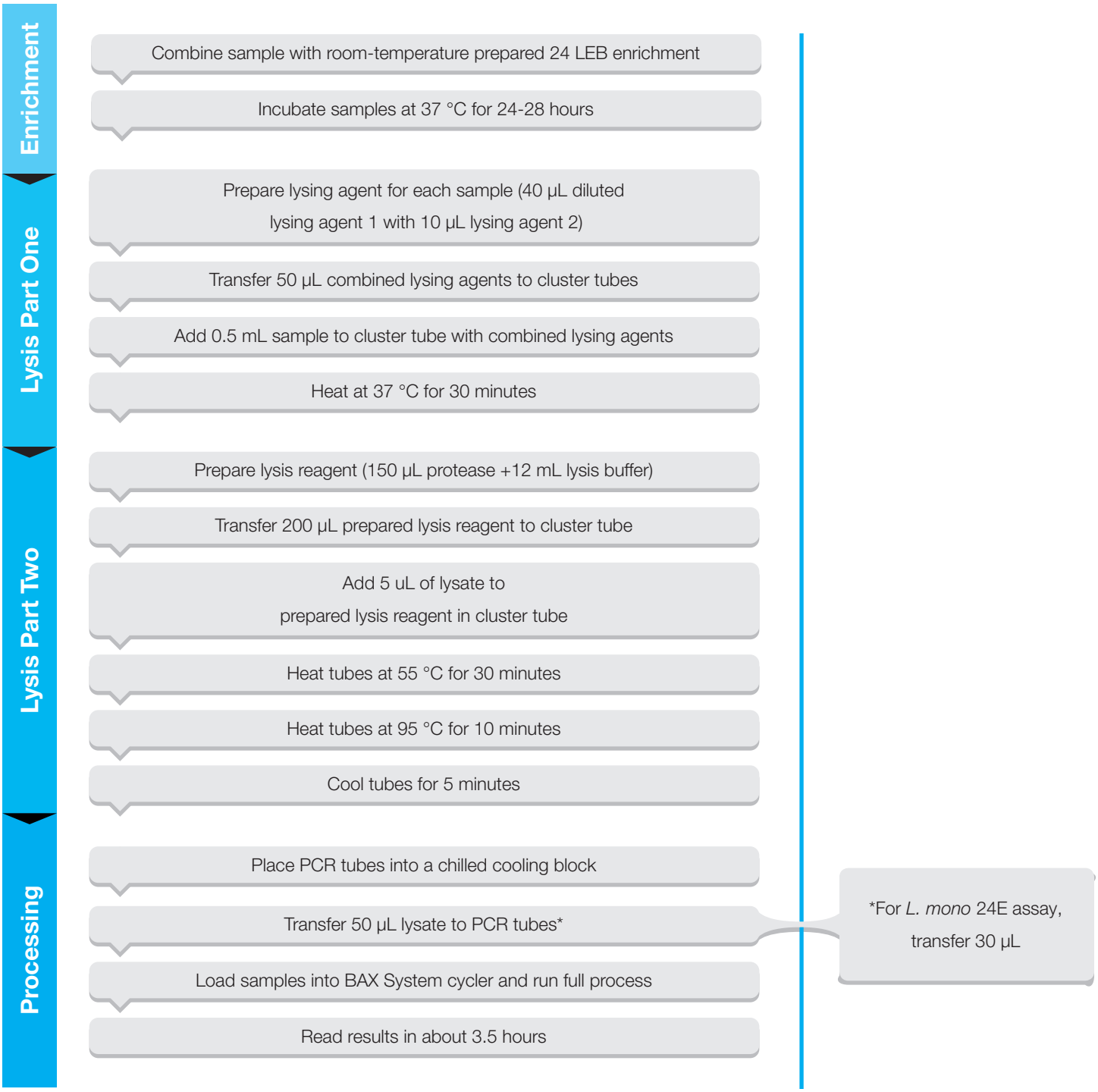
Protocol Overview Flowchart: Standard Assays



Before You Begin

Review all best practices, tips and techniques described in Chapter IV: BAX System Best Practices before beginning any BAX System protocol.

Protocol Overview Flowchart: 24E Assays



Before You Begin

Review all best practices, tips and techniques described in Chapter IV: BAX System Best Practices before beginning any BAX System protocol.

Protocol Overview Flowchart: Real-Time Assays

Enrichment

- Combine samples with pre-warmed enrichment media
- Incubate samples for the appropriate enrichment time
- If necessary, transfer sample to pre-warmed secondary enrichment media and incubate samples for the appropriate enrichment time

Sample Prep

- Prepare lysis reagent (150 µL protease + 12 mL lysis buffer*)
  - *For Real-Time Genus *Listeria* and *L. monocytogenes* assays, also add 200 µL of Lysing Agent 2.
- Transfer 200 µL of prepared lysis reagent to cluster tubes
- Add 5 µL* sample to cluster tube of prepared lysis reagent
  - *For Real-Time *E.coli, E. coli* EXACT and STEC assays, add 20 µL of the sample.

Lysis

- Heat tubes at 37 °C for 20 minutes *
  - *For Real-Time *Staph aureus* assay, heat tubes at 55 °C for 60 minutes.
  - *For Real-Time Genus *Listeria* and *L. monocytogenes* assay, heat tubes at 55 °C for 30 minutes.
  - Lysing steps can be performed using the automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.
- Heat tubes at 95 °C for 10 minutes
- Cool tubes for 5 minutes

Processing

- Place PCR tubes into a chilled cooling block
- Transfer 30 µL of lysate to PCR tube*
  - *For Real-Time *Salmonella* and *E. coli* EXACT assays, hydrated PCR tubes must sit in the cooling block for 10-30 minutes.
  - *An optional 10-30 minute hold of the hydrated PCR tablets in the cooling block is recommended for the Real-Time Genus *Listeria* and *L. monocytogenes* assays in order to be consistent with the Real-Time *Salmonella* procedure above.
- Load samples into BAX system cycler and run full process
- Read results in about 90 minutes

IV. BAX System Best Practices

Tips for Sample Prep Technique

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

Tips for Sample Prep Technique (Continued)

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

Tips for Sample Preparation

- According to your laboratory SOP, prepare one positive control, one negative control and/or media blank and run along with your selected samples.
- For re-testing purposes, enrichments can either be stored 4 to 5 hours at room temperature or up to 48 hours at 2 to 8 °C.
  For confirmation purposes only, BAX Real-Time assays for *Salmonella*, *L. mono* and Genus *Listeria* have all been NF-certified by AFNOR for primary enrichment holds of up to 72 hours for confirmations or retesting, or according to your laboratory SOP.
- When preparing enrichment broth, use deionized water that is compatible with PCR testing.
- Use a thermometer to ensure correct incubation temperature before preparing samples. Incubators should be within ± 2 °C of the specified temperature, unless you are following Health Canada and AFNOR Certifications standards which require an incubator capable of maintaining ± 1 °C.

Tips for Enrichment Media

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

A Note on Shaking Samples After Enrichment

After enrichment, samples *should not* be shaken before aliquots are removed for lysis. For many sample types, shaking samples after enrichment increases the chance that larger particulates are transferred to the lysate, which could inhibit the PCR process.

If samples have been agitated after enrichment, let sit for at least 10 minutes before transferring aliquots to cluster tubes for lysis.

Tips for Sample Lysis

- Use a thermometer to ensure set temperatures are correct and within the approved temperature stability ranges before heating samples (see “Note on Heating Block Temperature Stability” in Chapter I).
- When preparing lysis reagent, mix the protease and lysis buffer by capping the bottle and inverting 8-10 times. Do not over-mix lysis reagent, which can cause the mixture to foam.
- If desired, you can make smaller volumes of lysis reagent by keeping the ratio at 12.5 µL protease to 1 mL lysis buffer. Lysis reagent in capped tubes will remain stable for up to two weeks when refrigerated at 2 to 8 °C.
- After lysis has been completed, unopened lysate can be stored at 2 to 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. Stopping the protocol prior to the end of lysis is not recommended, as this may compromise the results for some sample types.
- For steps using a cooling block, minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible.
- Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.

Tips for PCR Tablet Hydration

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

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

V. BAX System Hardware

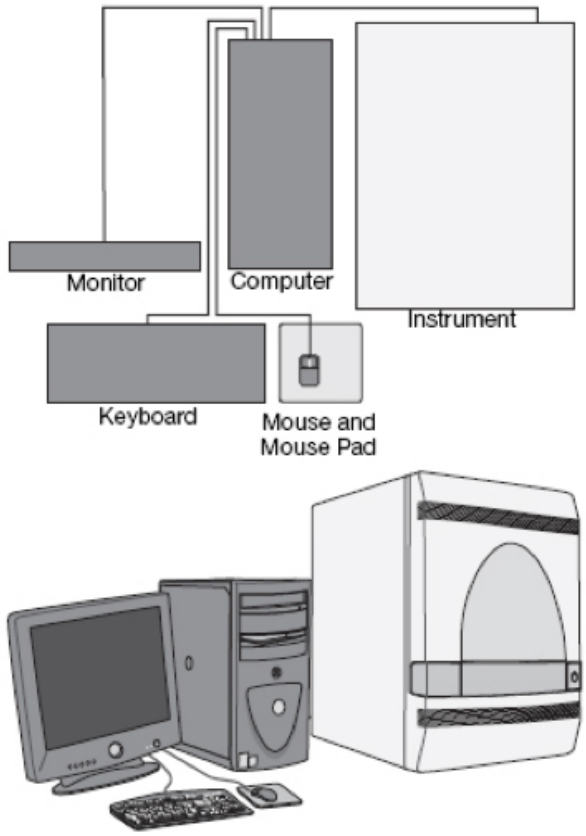
Installing the System

Before placing the system:

- Verify that the designated workbench supports at least 54.5 kg (120 lbs).
- Verify that the pathway to the final position of the instrument is clear of obstructions.
- Verify that each Q7 System has a dedicated 15 amp circuit to ensure proper functioning.
- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting. Coordinate your intentions with your assistant before lifting and carrying.
- Keep your spine in a good neutral position, bend at the knees and lift with your legs. Do not lift an object and twist your torso at the same time.
- When moving the computer, tilt the box on its side, then slide the contents out of the box.

To place the system:

1. Open the crate and remove any protective covers.
2. Lift and place the instrument onto the bench.
3. Unpack the computer boxes and place the monitor, computer, keyboard, and mouse on the bench as shown.



Suggested layout for BAX System components.

⚠ WARNING - PHYSICAL INJURY HAZARD

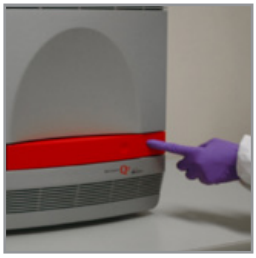
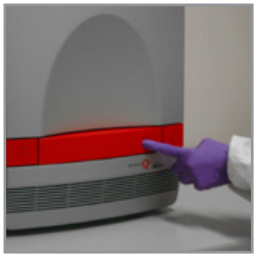
Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least 2 people are required to lift the instrument.

Connecting the System

Note: *To connect the BAX System components, you'll need a thin screwdriver to connect the power cord (included).*

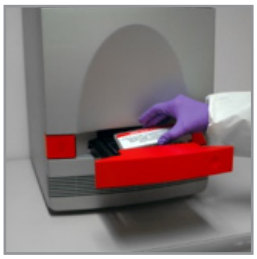
1. Connect the power cord to the instrument.
2. Connect the power cord to a receptacle wall circuit (or a UPS).


3. Press the power button and wait for the system to boot (about 30 seconds).
4. When the instrument displays the 'Power' status light, press the dimpled area on the right side of the drawer to open it.

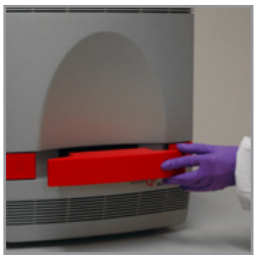
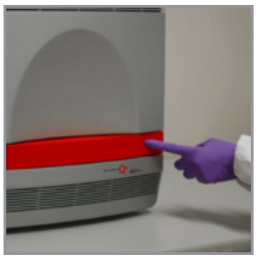
5. Remove the shipping plate from the drawer and set it aside.

Note: *Do not discard the shipping plate. It can be used to store or move the BAX System Q7.*

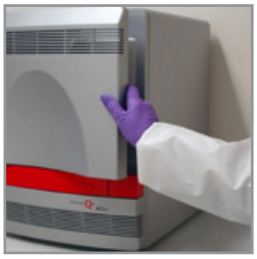



6. Close the drawer.
7. Press the power button to turn off the power to the instrument.

Note: *When closing the instrument drawer, apply pressure to the right side and at an angle.*

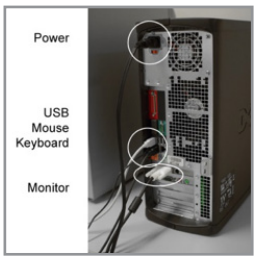
8. Insert a thin screwdriver or other thin, flat tool into the access hole (keyhole) located on the edge of the front panel (access door).
9. Carefully push the tool straight in to release the spring latch, allowing the panel (access door) to swing open.

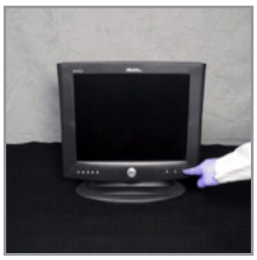
Connecting the System (Continued)

10. Make sure that the heating cover is pulled fully forward.
11. Close the access door of the instrument.

12. Connect the Universal Serial Bus (USB) cable to an available USB port on the computer.
13. Unpack and connect the mouse, the keyboard, and the monitor. Verify that all connections have been made correctly.



14. Power on the computer and monitor.



15. Connect the Universal Serial Bus (USB) cable from the computer to the back of the instrument.

Shutting Down the System

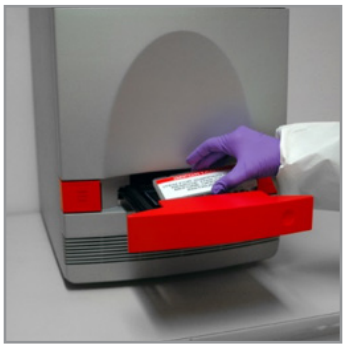
Short-term

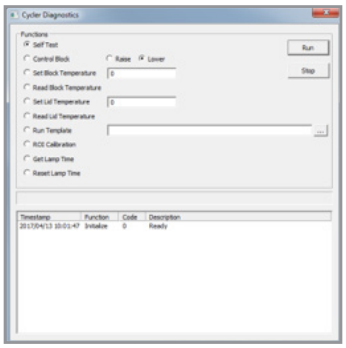
If you need to shut down the system for seven or fewer days, simply check that no rack is in the tray, then press the power button to power down the BAX System instrument.

Long-term

If you are shutting down the system to move, ship or store it, follow these steps:

1. Load the original shipping plate that came with the system into the tray. If you no longer have the shipping plate, use an unused rack plate.
2. Select DIAGNOSTICS > CYCLER from the menu bar. The Instrument Diagnostics window opens.
3. Select **Control Block** and **Raise** from the list of options.
4. Click the **RUN** button. The bottom pane of the window displays the status.
5. Power down the instrument.





This procedure allows the instrument optics block to rest upon the plate, protecting it during storage or shipment.

Note: *Do not turn on power to the instrument after performing this long-term shutdown procedure until the instrument is in place for use. Powering on the instrument lowers the plate, exposing the optics block to potential damage during storage or shipment. If you power on the instrument by mistake, make sure to repeat steps 2-5 for long-term shutdown.*

Performing Maintenance

The BAX System has been designed for ease of use and reliability. Careful design has created a system that does not require routine maintenance other than cleaning, as described below.

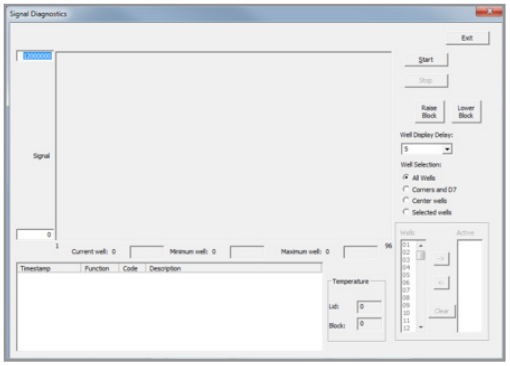
Routine maintenance

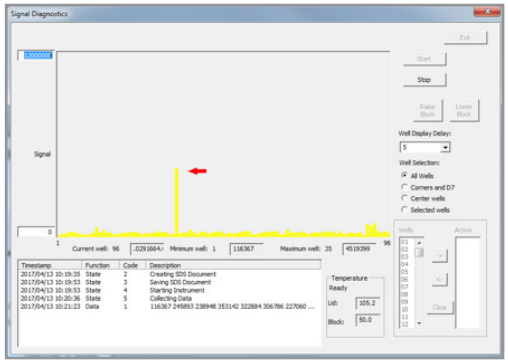
To prevent the accumulation of dust and debris wipe the instrument surfaces with a lint-free cloth. If needed, the casing can be wiped off with a damp cloth using mild soap or common laboratory disinfectant, such as 70% ethanol. To prevent moisture from getting inside the instrument, do not spray any cleaning solutions directly on the instrument casing. Do not use cleansers containing iodine or acetone.

Cleaning the sample block

First, use the Well Signal Diagnostic to determine if the sample block should be cleaned. The Well Signal Diagnostic should be run if the background dye calibration plate fails, or according to the standard operating procedures of your laboratory. The results of this diagnostic allow you to see which wells are producing strong background signal – these are the wells that should be cleaned. Then, follow the steps below to clean the sample block with deionized water.

Note: Do not use bleach to clean the sample block.

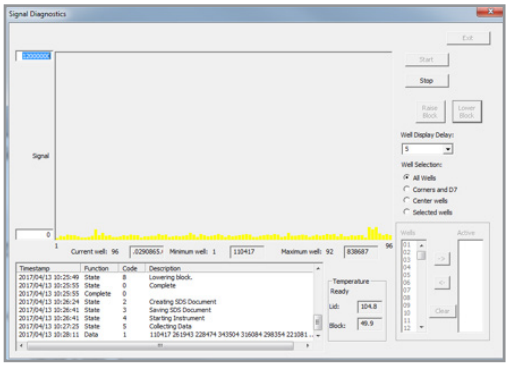




Part 1. Using the Well Signal Diagnostic

1. Make sure there are no samples or PCR tubes in the cycler/detector.
2. From the menu bar, select DIAGNOSTICS > WELL SIGNAL to open the Signal Diagnostics window.
3. Click the **START** button to run the Well Signal diagnostic. The results will display in the Signal Diagnostics window when the test is complete.
4. If one or more wells show a signal significantly higher than the other wells (see the example to the left), then the sample block should be cleaned. Click the **LOWER BLOCK** button to lower the sample block, then follow the procedure described below.

Performing Maintenance (Continued)



5. If the signal appears relatively low and uniform across the wells, the sample block does not need to be cleaned at this time.

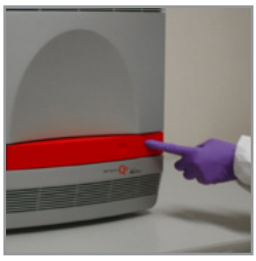
Note: *To save the results of the Well Signal Diagnostic, press the Print Screen ("PrtScn") button on the computer keyboard to create a screen capture of the results. Then, launch a program such as Microsoft® Word or Microsoft® Paint and select the Paste option to save the screen capture.*

Part 2. Cleaning the Sample Block

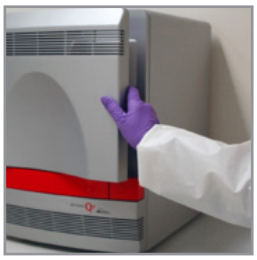
⚠ WARNING - PHYSICAL INJURY HAZARD

During instrument operation, the sample block can be heated as high as 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

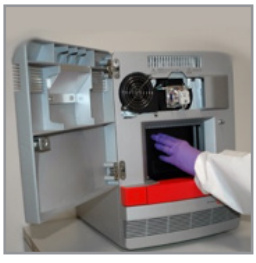
1. Power down and unplug the BAX System instrument. Allow to cool for 15 minutes.

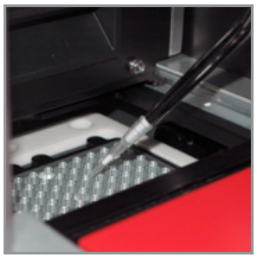


2. Insert a thin screwdriver or other thin, flat tool into the access hole (keyhole) located on the edge of the access door.
3. Use the tool to carefully release the spring latch, allowing the panel (access door) to swing open.



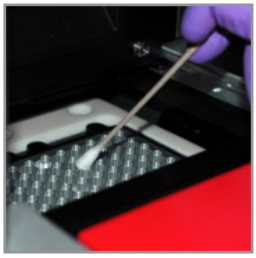
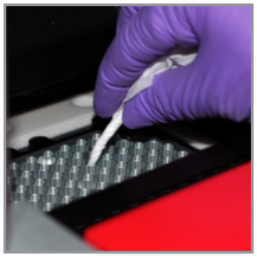
4. Push the heated cover door to the back of the instrument.
5. Pipette a small volume of deionized water into each well (small hole). Let sit for 5 minutes.





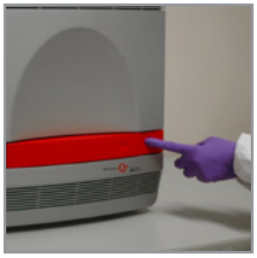
Part 2. Cleaning the Sample Block (Continued)

6. Scrub the inside of each well with a CleanFoam® swab (ITW Texwipe P/N TX751B)
7. Absorb the excess water with a lint-free cloth or a dry swab
8. Re-run the Well Signal Diagnostic described above to determine if the sample block has been cleaned sufficiently.
   a. If the block is clean, proceed to step #9.
   b. If the block is not sufficiently clean, repeat the "Cleaning the Sample Block" procedure using 95% ethanol instead of deionized water. Make sure to rinse the wells thoroughly with deionized water to remove all traces of ethanol before re-running the Well Signal Diagnostic.

9. Pull the heated cover door to the front of the instrument, and close the access door


10. Plug in and power on the BAX System instrument

Changing the Fuse

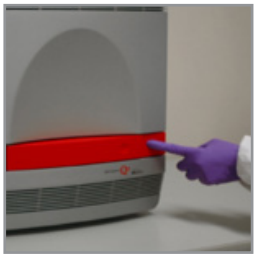
If the fan does not run, or the power indicator does not illuminate, or the halogen lamp does not work after replacing the bulb, a fuse may have to be replaced. You'll need a flat-head screwdriver to remove the fuse holders.

CAUTION - FIRE HAZARD

For continued protection against the risk of fire, replace fuses only with Listed and Certified fuses of the same type and rating as those currently in the instrument.

Changing the Fuse (Continued)

1. Power down and unplug the BAX System instrument. Allow to cool for 15 minutes.



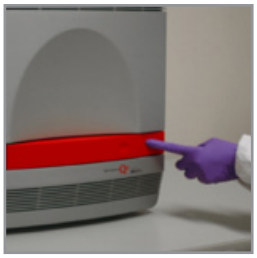

2. Using a flat-head screwdriver, unscrew and remove fuse holders from back of instrument.

3. Remove each fuse from its holder and inspect for damage. Carbon typically coats the inside of failed fuses (on left).


4. Replace failed fuse with a new one (12.5 A, 250 V, 5x20 mm), then replace fuse holders in instrument.

5. Plug power cord into receptacle and power on the instrument. Installation is successful if unit powers on.

Note: *Fuse failure can be the result of fluctuations in the supplied power to the instrument. To prevent further failures, consider installing an electrical protective device*

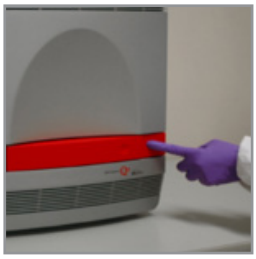



Changing the Halogen Bulb

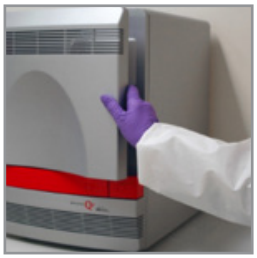
The bulb in the halogen lamp should be replaced after approximately 2,000 hours of life. After changing the halogen bulb, you must perform a dye calibration to re-calibrate the instrument and create a new calibration file. Follow the steps described in the next section, “Calibrating the Instrument”, to complete this process.

Note: *The quality of replacement bulbs can vary widely. For warranted results, use only bulbs that have been tested and approved for use with the BAX System. You can order approved replacement bulbs from Hygiena (Part No. MIS2001).*

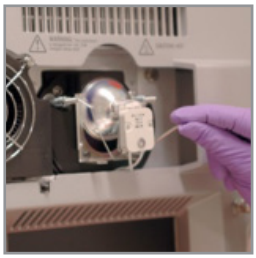
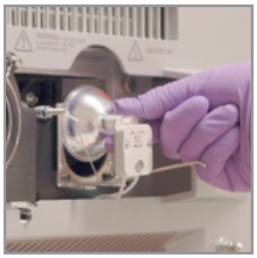
1. Power down and unplug the BAX System instrument. Allow to cool for 15 minutes.



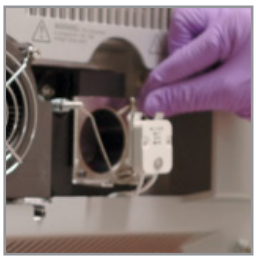
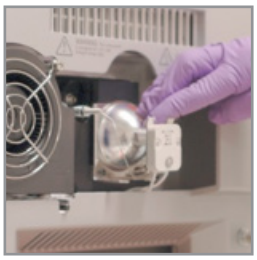

2. Insert a thin screwdriver or other thin, flat tool into the access hole (keyhole) located on the edge of the access door.
3. Use the tool to carefully release the spring latch, allowing the panel (access door) to swing open.

4. Pull the lamp release lever forward to release the halogen bulb from its mount.
5. Firmly grasp the failed bulb and lift it up and out of the slotted mount.

6. Push the lamp release lever back into the upright position.
7. Insert a replacement bulb firmly into the slotted mount and slide it down into place.

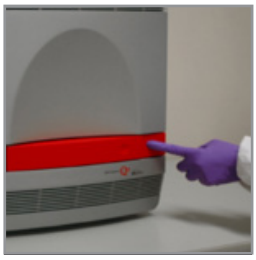



Changing the Halogen Bulb (Continued)

8. Close the access door, then plug the power cord back into the receptacle.
9. Power on the system.

10. Check that light is visible behind the grill on the front panel by selecting DIAGNOSTICS > CYCLER > SELF TEST from the menu bar, then click the RUN button.
11. Select DIAGNOSTICS > CYCLER > RESET LAMP TIME from the menu bar, then click the RUN button.

Note: *We recommend that you re-calibrate the instrument after changing the halogen bulb.*



Calibrating the Instrument

Note: *Calibration is important when running real-time PCR assays. Verify you have the correct dyes loaded when running assays. Contact technical support if you have any questions.*

BAX System Q7 instruments are calibrated prior to shipment. The calibration file is included on a removable storage device that is attached to the back of the instrument for backup. Each time you reinstall the BAX System application, **you must back up your calibration file** to safeguard against it being overwritten or damaged during the reinstallation process. See Chapter VI: BAX System Software for more details.

You must calibrate the BAX System Q7 instrument under the following circumstances:

- The calibration report does not include the desired assay in the list of calibration files
- You have changed the halogen bulb
- Repairs were made to the instrument by a non-Hygiena repair facility. and a new calibration file must be created

Note: *To ensure that calibration is performed successfully, clean the sample block and wells before performing a dye calibration. See "Cleaning the Sample Block" for instructions.*

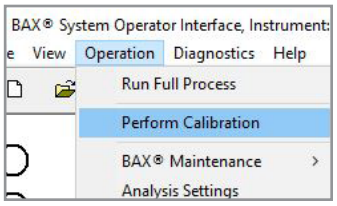
Calibration Kits

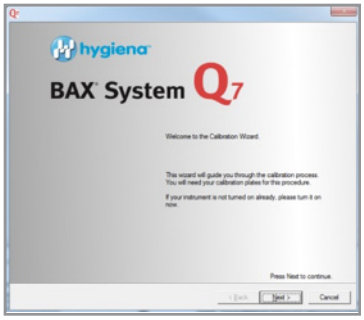
Calibration kits are available directly from Hygiena (Part No. KIT2026).

Note: A HEX Calibration kit may also be required to run certain foodproof® assays. (Refer to Product Instructions for KIT230340 and the instructions for use for the assays).

Kits should be stored at -15 °C to -25 °C in the original foil envelope to protect it from exposure to light. Before use, bring the plates to room temperature and check that the plates are clean and the liquid levels are even across all the wells. For best success in calibration, the plates should be thawed by unpacking from the kit and laying each plate on a flat surface. Calibration kits can typically be used multiple times as long as they are kept frozen when not in use.

1. From the OPERATION menu, select PERFORM CALIBRATION. The **Calibration Wizard** screen appears.
2. Make sure the Q7 instrument is powered on and click **NEXT** .

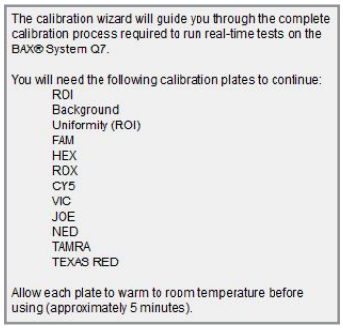




3. The next screen lists all the calibration plates needed to complete all the prompts of the calibration wizard.

   Click **NEXT** .

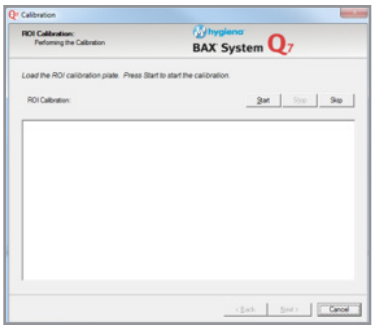
***Note**: The dyes listed and the order in which they are listed may vary depending on the BAX® Q7 software version in use.*



***Note:** Plate order may vary during calibration.*

Calibration Kits (Continued)

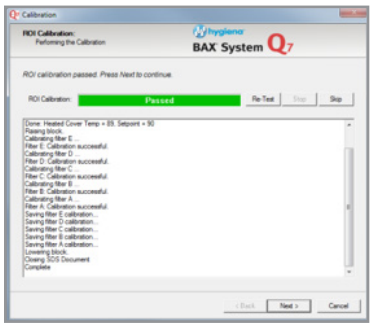
4. At the **ROI Calibration** screen, load the ROI plate and click the **START** button at the top of the window. The window displays a status bar and remarks on calibration progress.



Note: *Make sure calibration plates are at room temperature before use.*

Note: *Check plates before use. If there are droplets on the top film, flick the plate so drops return to the wells. If one or more wells have significantly less liquid, the calibration kit should be replaced.*

5. At the end of ROI calibration, the status bar displays a green “Passed” result. Click **NEXT** . At the prompt, load the **Background** plate and click **START** . Repeat this procedure at each prompt until all eleven calibration plates have passed.

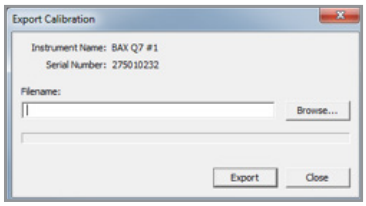


6. When you click **NEXT** on the last plate, the final screen appears. Click **FINISH** to exit the wizard.

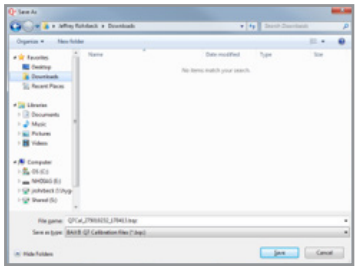
Note: *Make sure to remove the last calibration plate*

Calibration Kits (Continued)

7. Back up your calibration files to external media in case you need to restore them. From the OPERATION menu, select BAX MAINTENANCE > EXPORT CALIBRATION.



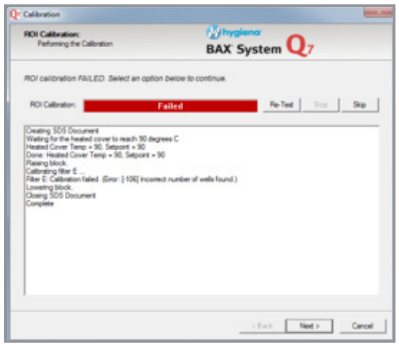
8. Click the **BROWSE** button to navigate to the folder where you want to store the file. The system suggests a name for the file based on the date.

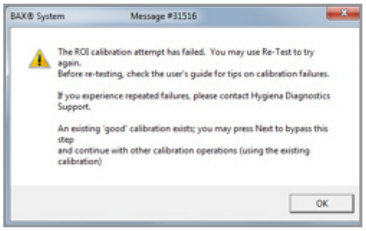


Calibration failure

If the plate does not pass calibration, the status bar displays a red “Failed” result.

A message box automatically appears that explains your options to re-test or continue with calibration using the last successful calibration file for that plate. Click **OK** to continue.





For tips on troubleshooting problems with calibration, see Appendix D: Troubleshooting.

Customized PPH Plate

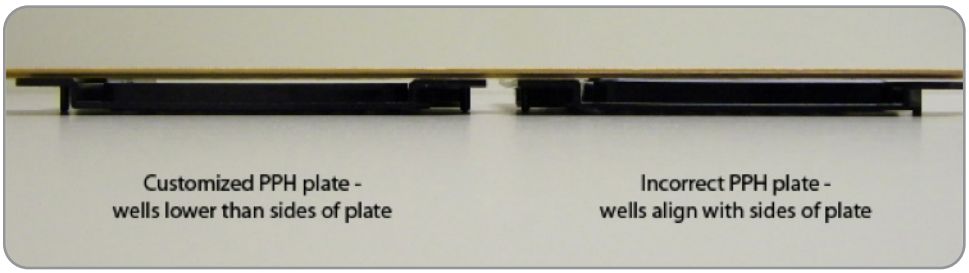
The BAX System Q7 instrument is supplied with a customized PPH plate - the plastic rack inside the Q7 instrument drawer. The modifications made to this plate from the manufacturer's version provide for tighter seating of the PCR tubes included in BAX System kits, which is needed for optimal performance.

If your lab also uses other thermal cyclers that are similar to the BAX System Q7 instrument, there is a potential risk of confusing the PPH plates in each unit. Using a non-customized PPH plate in the Q7 instrument could interfere with performance. We recommend that whenever you clean the Q7 units or have them serviced, check that the correct customized PPH plate is in place before use.

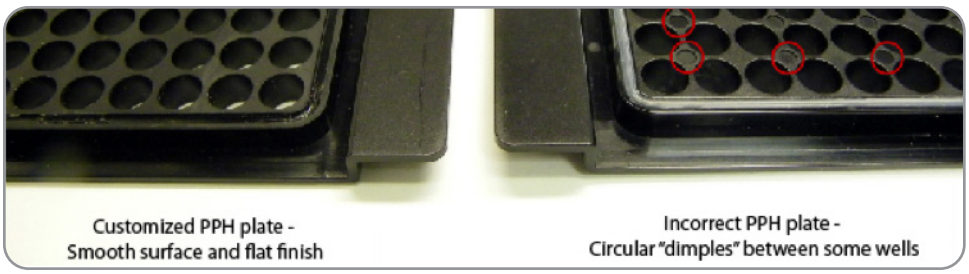
Modifications to the PPH Plate

To ensure that the correct customized PPH plate is used with your BAX System Q7 instruments, check that the PPH plate contains the following features:

1. **The customized PPH plate does not have a rubber gasket around the top perimeter of the wells.** The incorrect PPH plate may have a clear or white-tinted rubber gasket around the wells.



2. **The wells of the customized PPH plate have been milled down to be shorter than the sides of the plate.** The incorrect PPH plate has a well height that aligns with the sides of the plate.

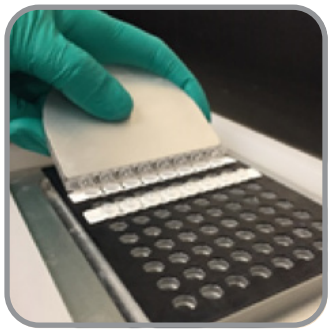


3. **The surface of the customized PPH plate is smooth and has a flat (not shiny) finish.** The incorrect PPH plate has a shinier finish and has circular "dimples" between some of the wells.

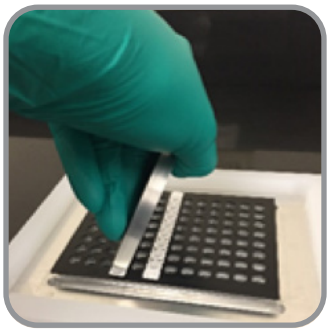
Capping/Decapping Tools

With your purchase of the BAX System start-up package, you receive a tool* that facilitates decapping and resealing the PCR tubes. This tool allows you to 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).

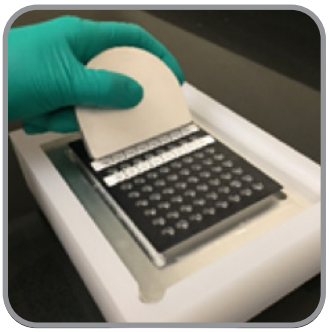
A. Removing Domed Caps from PCR Tubes



Place tool flange behind row of caps.

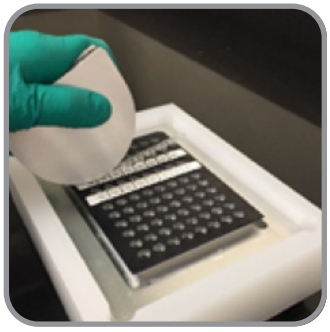


Tilt the tool forward and push down so that it locks over the caps.

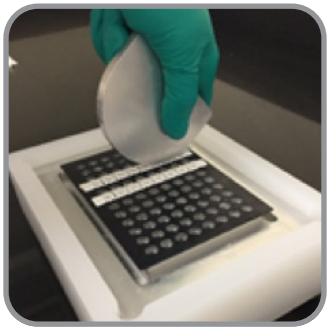


Tilt the tool backward so that the caps lift off.

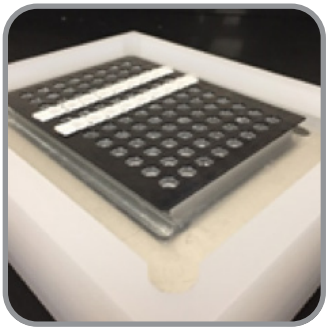
B. Sealing PCR Tubes with Flat Optical Caps



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

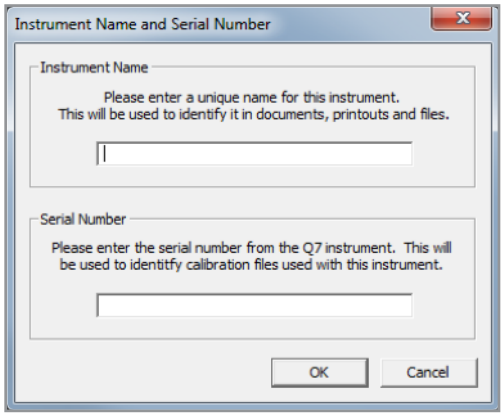
Shipping Instructions

In the event your BAX System instrument needs service, please contact Hygiena Diagnostics Support to obtain a return authorization number and instructions for returning the unit:

- Thoroughly clean the unit of any biohazardous substances.
- Shut down the system for shipping, as described under long-term shut down procedures in this user guide.
- To avoid damage to the instrument, use ONLY the Hygiena-supplied loaner/replacement container for shipping the unit needing service.
- Ship the unit to the address on the return form.

VI. BAX System Software

The first time you open the application, you are prompted to enter a name and a serial number for the BAX System instrument to which the computer is connected. The name appears in the title bar of all windows, in files and on printed reports.



To change the instrument name later, select OPERATION > BAX MAINTENANCE > UPDATE INSTRUMENT NAME AND SERIAL NUMBER.

You can run several copies of the BAX System application concurrently. This is useful for reviewing data on other files while the instrument is performing PCR and analysis on a rack of samples.

The first instance of the BAX System application normally opens in standard mode (operator interface), which provides all the features necessary for operating the instrument. Any concurrent copies of the application are restricted to data analysis mode (analysis interface) and do not allow for operation of the instrument.

Window Sections

The main window is divided into these sections:

Title bar – shows the currently open rack file and the instrument name. When you are creating a new file, the title bar displays “untitled” until you save it with a name.

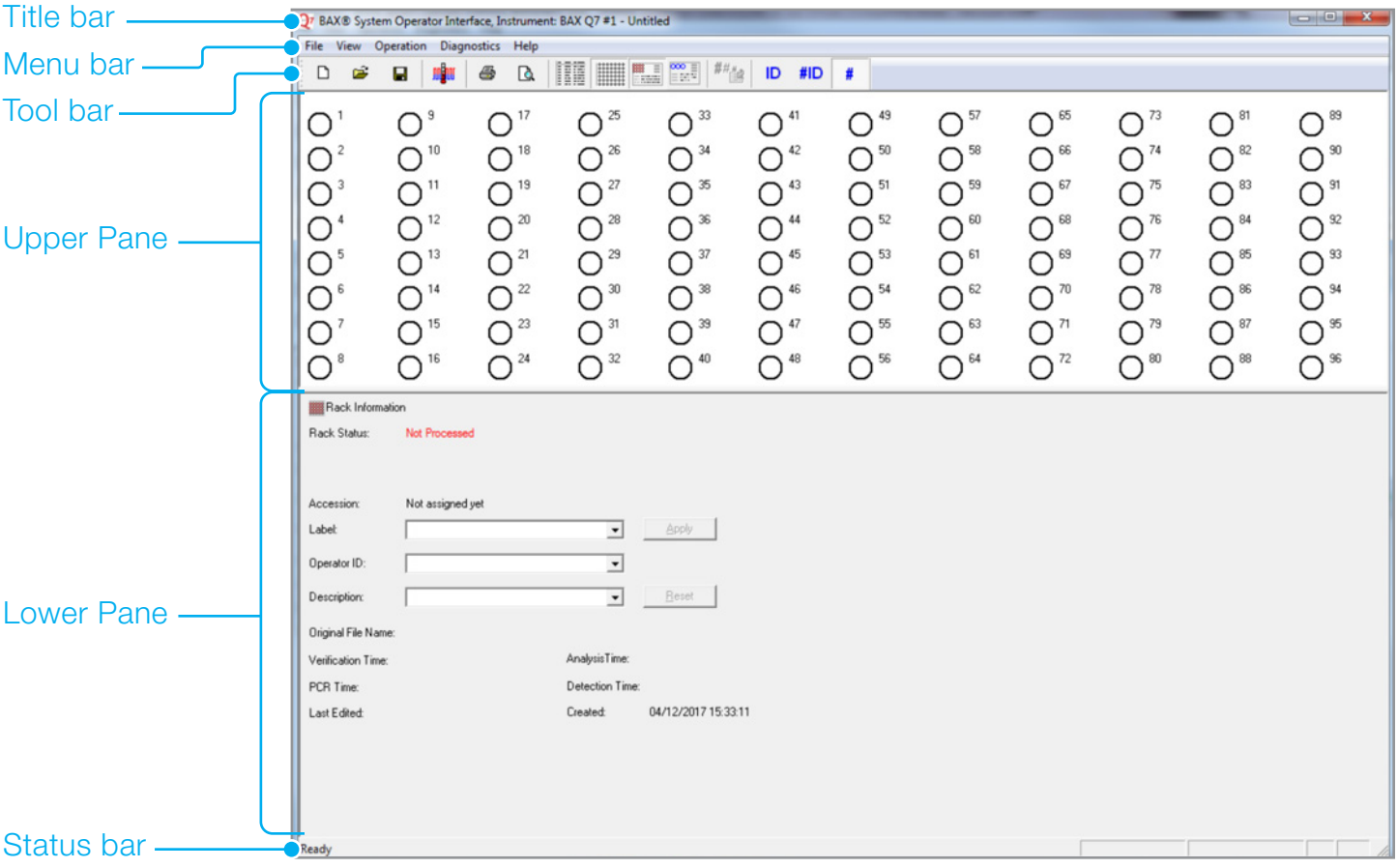
Menu bar – contains the main functions that you use to enter data, edit and otherwise manipulate the file.

Tool bar – displays icons of “shortcuts” for some of the common functions also found in the menu bar.

Upper pane – displays either a graphical layout that corresponds to the PCR tube rack when the grid icon in the tool bar is selected or a detailed report when the list icon is selected.

Lower pane – displays a form with fields for entering data. These fields change when you switch from “rack” view to “well” view.

Status bar – displays status messages, including explanations for the icons on the tool bar.



BAX System application window elements

Menu Bar

FILE Menu

Creates, opens and saves your rack file. A rack file contains all the information you enter on a batch in rack view, on the individual samples in well view, and the test results.

NEW creates a blank file. If the current rack file has been edited, you are prompted to save it before the application creates a new file.

OPEN displays the standard Windows dialog box for navigating to the desired file. Files saved in this application use a ".bax" file extension.

SAVE writes the current file to disk.

SAVE AS allows you to save the current file under a different name.

RACK WIZARD guides you with screen prompts in creating a new rack file or importing an existing file.

EXPORT FILE allows you to save the file as delimited text to your folder of choice.

EXPORT CSV RESULTS allows you to export the data as a CSV file to your folder of choice.

CONFIGURE EXPORT provides options for automatic export and for which data fields should be included in the text file.

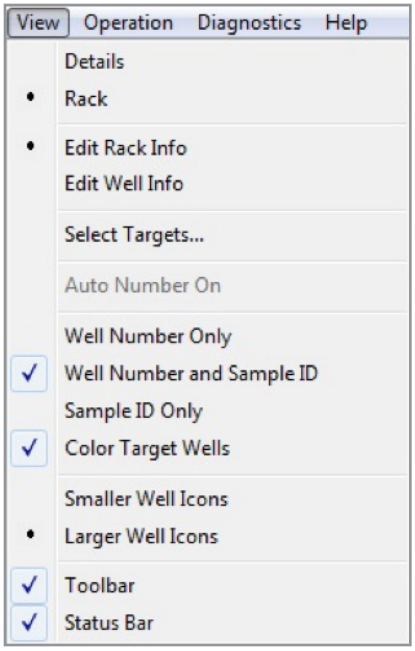
EDIT PASSWORD allows administrators to set passwords and users to change their passwords (available only if this security option was selected at installation – see "Security Options" in Chapter VI: BAX System Software).

PRINT, PRINT PREVIEW and **PRINT SETUP** perform according to the standard Windows methods.

CLEAR RECENT FILE LIST allows you to remove the list of recent files that appear in this section after use.

RECENT FILE is a placeholder for the list of recent files that appear in this section after use.

EXIT quits the entire application without asking for confirmation.



VIEW Menu

Allows you to switch among various views.

DETAILS displays all of your test results as a list in the upper pane.

RACK displays a graphical grid of the wells in the upper pane.

EDIT RACK INFO and **EDIT WELL INFO** display the data fields for rack or well information in the lower pane.

SELECT TARGETS opens a dialogue box that lets you define the targets in the drop-down menu list.

AUTO NUMBER ON toggles with **AUTO NUMBER OFF** to allow for customized numbering sequences.

WELL NUMBER ONLY displays the well numbers.

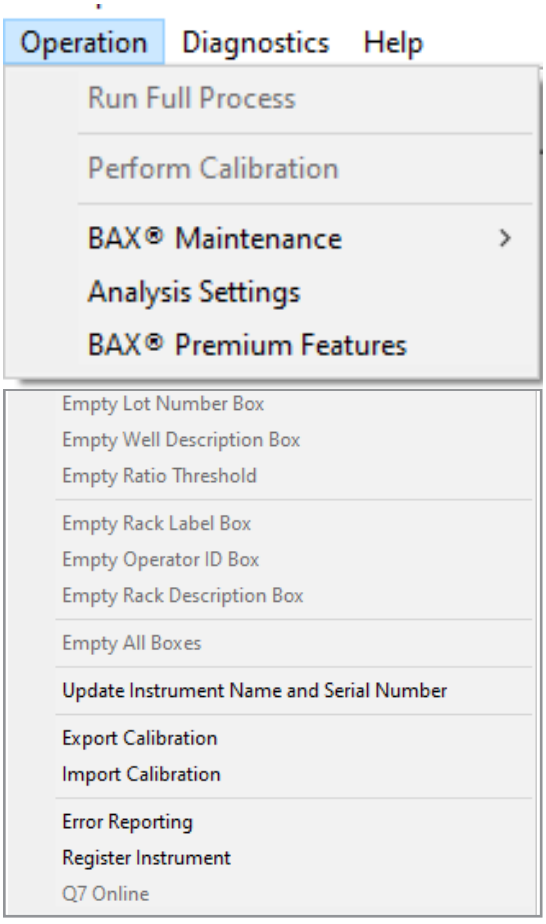
WELL NUMBER AND SAMPLE ID displays the well numbers and the ID you have assigned to each.

SAMPLE ID ONLY displays the wells with the ID you have assigned.

COLOR TARGET WELLS displays a target-specific colored box around each well.

SMALLER WELL ICONS and **LARGER WELL ICONS** toggle to adjust the size of your icons and font for longer label names.

TOOLBAR and **STATUS BAR** can be selected to display or deselected to remove from the window display.



OPERATION Menu

Allows you to process your samples and check the data.

RUN FULL PROCESS launches the PCR wizard for amplification and detection.

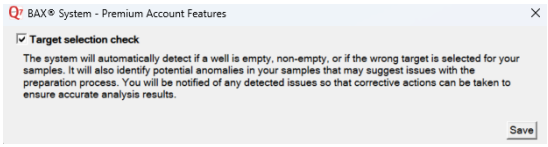
CALIBRATION launches the Calibration wizard that allows you to calibrate the instrument.

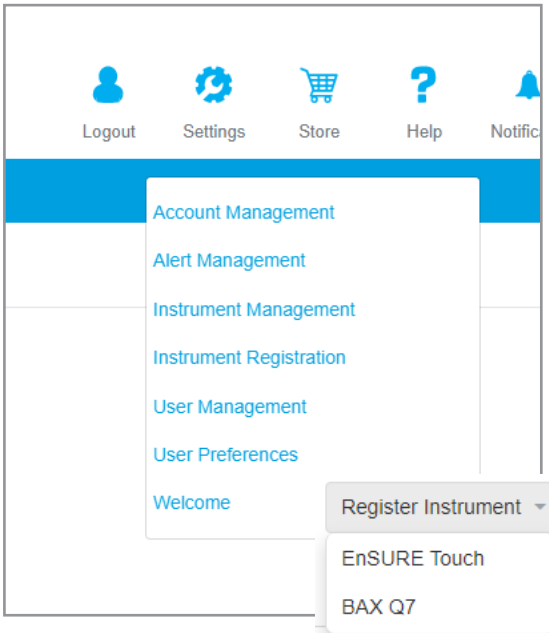
BAX® MAINTENANCE displays a submenu that allows you to clear the drop-down boxes on the lower pane of historical data, update the instrument name and serial number, import/ export calibration files and register the Q7 instruments Online.

ANALYSIS SETTINGS launches an analysis settings window that allows you to select Experimental algorithms to assist with result interpretation for select assays.

BAX® PREMIUM FEATURES launches a window to enable features available to users with a Premium Account via SureTrend.

A key feature is 'Target selection check' which can help identify empty wells, wrong target or sample anomalies.





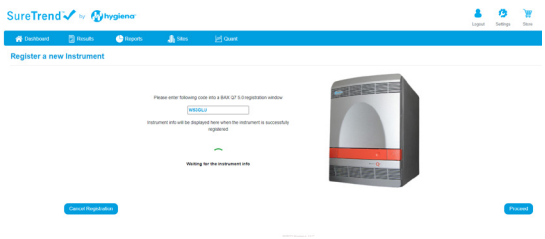
BAX Maintenance

Register Instrument

Allows you to create a SureTrend account if your company does not already have one. This will take you to the SureTrend software interface.

Under 'Settings' go to 'Instrument Registration'

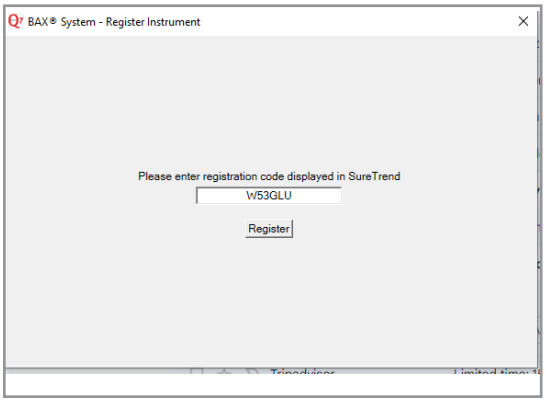
Under 'Register Instrument' dropdown, select 'BAX Q7' to register an instrument.



Register Instrument

Screen is redirected to SureTrend software registration page. Copy the code provided.

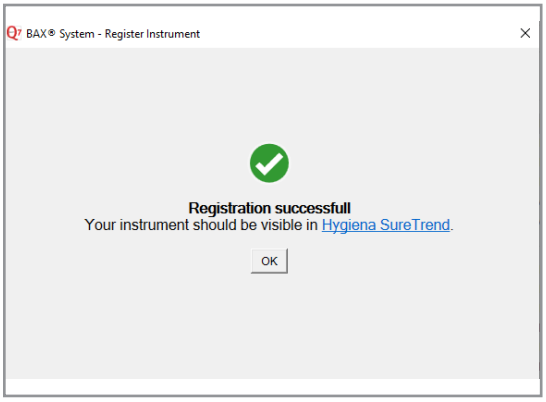
Entered the SureTrend code into the BAX Q7 System software and click the 'Register' button.



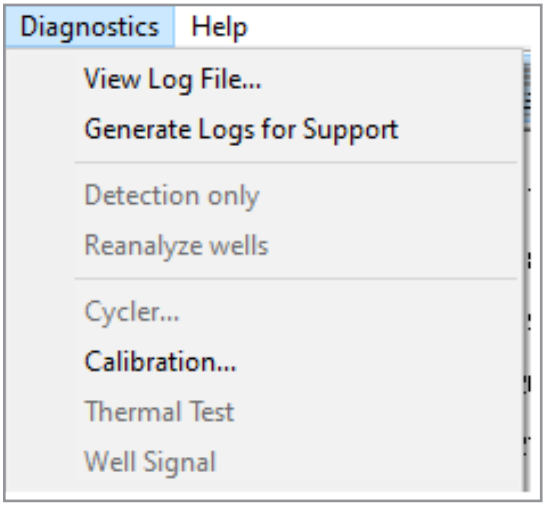
Registration Successful is displayed with the message that "Your instrument should be visible in Hygiena SureTrend".

Click OK to begin using the BAX System Q7 instrument.

Results of future PCR runs are automatically visible in Sure Trend Cloud.



Q7 Online Use this feature to enable an automatic upload of the BAX data to your SureTrend account when analysis completes. Also has the ability to enable mapping of well description as SureTrend location during automatic file upload.

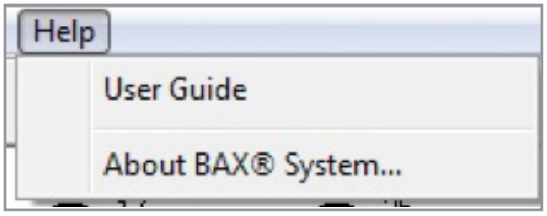


DIAGNOSTICS Menu

Provides for **VIEW LOG FILE, GENERATE LOGS FOR SUPPORT, DETECTION ONLY** and **REANALYZE WELLS** features.

Allows access to **CYCLER, CALIBRATION, THERMAL** and **WELL SIGNAL** diagnostics, including self-test on the instrument.

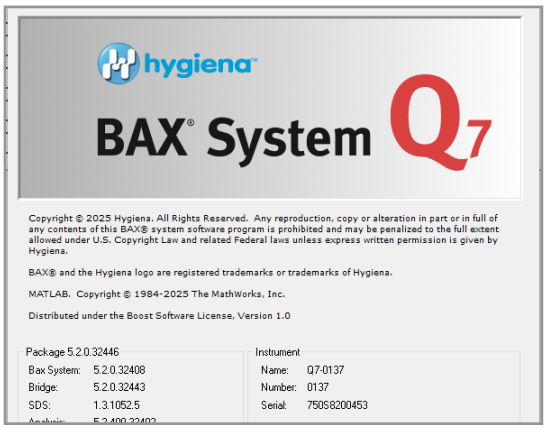
For Calibration Diagnostics, see “Troubleshooting Calibration” in Appendix D: Troubleshooting. For other tests, call Hygiena for instructions.



HELP Menu

USER GUIDE links to a PDF version of the user documentation that is installed with the application.

Note: *The first time you use this, you can use the installed Adobe Acrobat Reader to open the file. Microsoft Edge will also open the file, if installed on your computer.*



ABOUT BAX SYSTEM displays information about the current version of the application software, along with the instrument name, copyright and trademarks.

Tool Bar

The tool bar provides you with a series of quick-access buttons for common software features and commands.

	Create a new file
	Open an existing file
	Save the current file
	Run full process
	Print the current file
	Print preview
	Switch between the Details View and Rack view in the upper pane
	Switch between the Rack Information or Well Information view in the lower pane
	Auto number on/off
	Switch to display sample IDs only, well numbers with sample IDs, or well numbers only

Security Options

Your BAX System startup package is shipped with the BAX System application pre-installed on the computer. By default, the options for password protection and locked workstation are not enabled, thus allowing all users to launch the application and to use the workstation while samples are being processed.

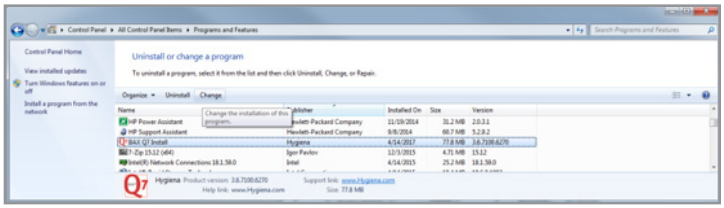
With password protection enabled, BAX System passwords are required to launch the application. In addition, the workstation is automatically locked after a run has begun. This prevents canceling the run or otherwise interacting with the workstation while a run is in progress, unless unlocked via password.

Note: *BAX System passwords are entirely separate from the passwords you use for the Windows® operating system. Before you can launch the BAX System, you need to access the desktop by logging in with a Windows® password.*

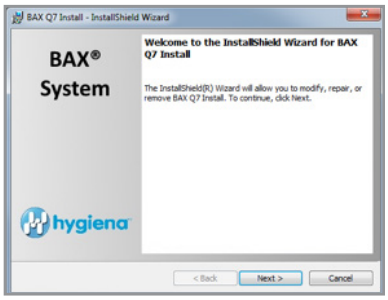
Modify the Installed Program

To enable the security features, you need to modify the BAX System application, as described below.

1. After closing all windows and files, click the START button and select CONTROL PANEL > PROGRAMS AND FEATURES. A window appears that lists all currently installed programs.



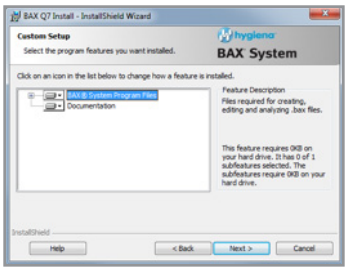
2. Scroll down the list to the BAX System application and click the **CHANGE** button. This launches the Install Shield wizard. Click the **NEXT** button.



Modify the Installed Program (Continued)

3. Select **MODIFY** from the list of options in the Welcome window, then click the **NEXT** button. The Select Features window appears.

4. ClicK **NEXT** to modify the BAX System program files.

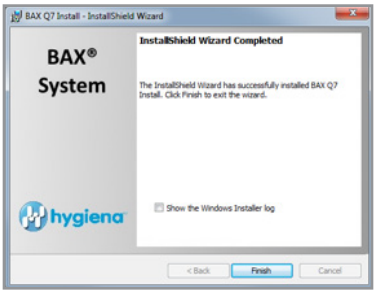


5. A window appears with the enable password option. Select “Enable Password Protection”.

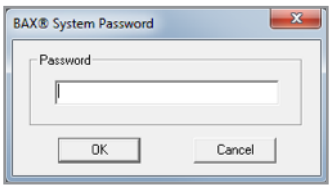
Modify the Installed Program (Continued)

6. Enter a password for the BAX System application, and click **NEXT** .

7. Follow the wizard prompts to finish the maintenance. You do not have to restart your computer for the security features to take effect.



8. Each time you launch your BAX System application, you will be prompted to enter a password.



Passwords

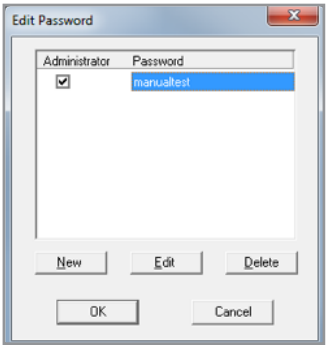
The person who sets the first BAX System password is the administrator. If all the users in your lab share a single password, the administrator needs to tell them the BAX System password so that they can launch the application and unlock the workstation during a run, if necessary.

Creating Additional Passwords

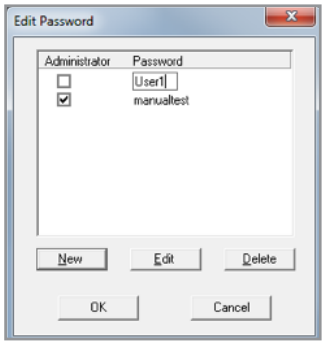
The administrator can create one or more additional BAX System passwords for other users without administrator permissions, as follows:

1. After launching the BAX System application with the established password, select FILE > EDIT PASSWORD from the menu bar. A window appears with the administrator box checked and its associated password visible

   This window is only available to administrators. It allows you to create BAX System passwords for additional users, edit and delete them or give them administrator status.



2. Click the **NEW** button to create a second user. The Administrator box and the Password box are blank. Enter a password and click **OK**. Repeat this step for as many passwords as are needed.



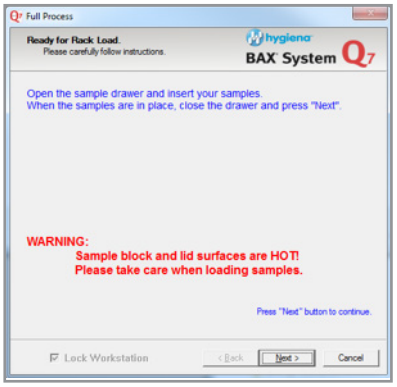
Changing Passwords

1. Users without administrator status can change their BAX System password by launching the application under the password created for them by the administrator, then selecting FILE > EDIT PASSWORD from the menu bar. The Edit Password window appears.

2. Enter the BAX System password assigned to you by the administrator in the old password field, then enter a new password, verify it and click **OK**. Each time you launch the BAX System application, you can use your newly defined password.

Locked Workstation

With password protection enabled, the workstation automatically locks while amplification/detection is in progress on the instrument. The Ready for Rack Load window displays a checkbox with “Lock Workstation” message at the bottom.



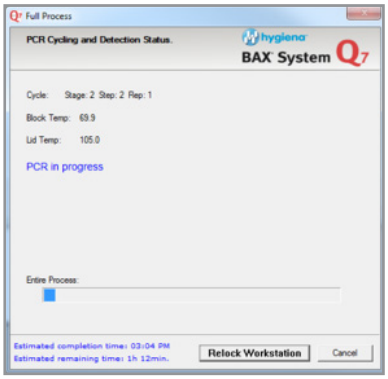
Click the **NEXT** button to begin amplification. A “Workstation locked” message travels around the window to remind you of this status.

If you move the mouse or touch any key after amplification has begun, a blank background covers the entire screen.

To unlock the workstation, enter the BAX System password that was used to launch the application, and click **OK** .

The amplification/detection status windows reappear, but the workstation is now unlocked. At this point, you can cancel the run or open other applications.

To re-lock the workstation, click **RELOCK WORKSTATION** button.

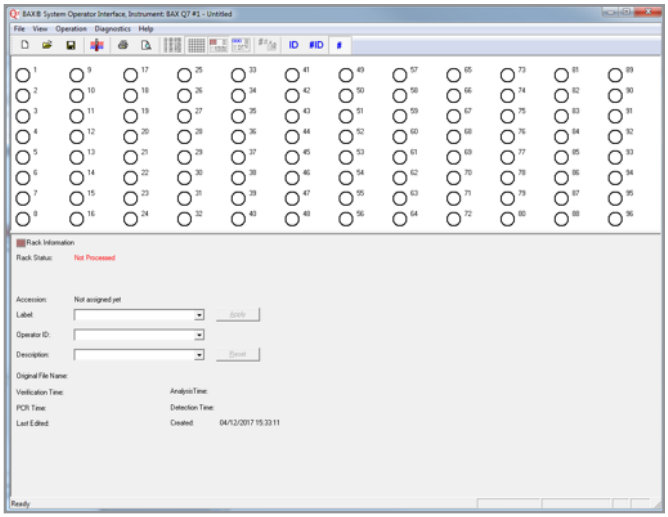


Creating a Rack File

1. Launch the BAX System application from the shortcut on the desktop.

   Note: *If you are using security options, you are prompted to enter a password. See "Security Options" in Chapter VI: BAX System Software for more information*.

2. The screen displays a window with an empty rack view. This window is divided into an upper pane, which displays a grid of 96 blank wells and a lower pane with fields for data entry. The lower pane changes when you switch between "rack" and "well" view, as described in the following sections.



Empty rack view

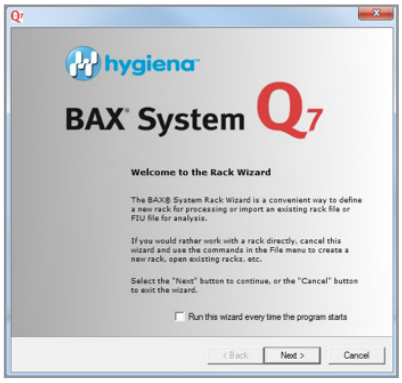
The rack file contains all the information on your samples that is required for analysis. You need to create this file and enter some information before loading your samples and running the program.

3. Select FILE > RACK WIZARD to create a rack file using screen prompts. You can also choose to enter the information manually.

Create a Rack File with the Rack Wizard

Select FILE > RACK WIZARD from the menu bar. You can choose to continue with the wizard by clicking **NEXT** or define a rack manually by clicking **CANCEL** .

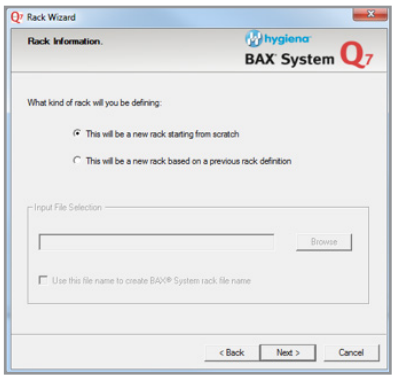
Note: *Use the checkbox at the bottom of the window to automatically run the rack wizard at startup.*



Choose the type of rack file you want to define:

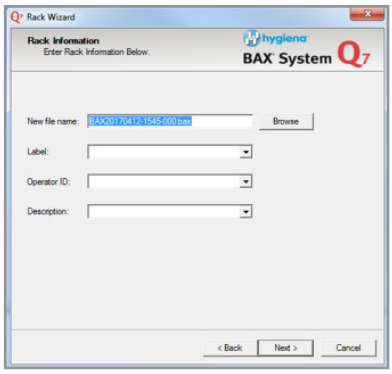
- New
- Based on a previous rack

Click **NEXT** to continue



Enter File Name, Label, Operator ID and Description. A file name based on the date is automatically generated, which you can change at this time.

Click **NEXT** to continue.



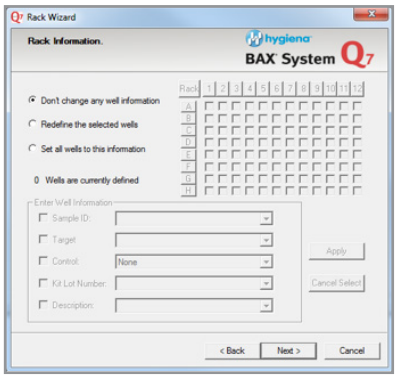
Create a Rack File with the Rack Wizard (Continued)

Choose how you want to define the information on your samples:

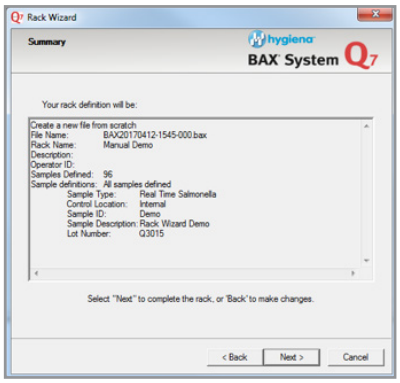
- Don't change any information
- Redefine selected wells
- Redefine all 96 wells

You can select wells by clicking on a row, a column or individual wells in the grid. Under "Well Information," you must select the Target from the drop-down menu. Other fields allow you to enter the Kit Lot Number, Sample ID and Description, if desired.

Complete the data fields, then click **APPLY**. When all sample information has been applied, click **NEXT** to continue.

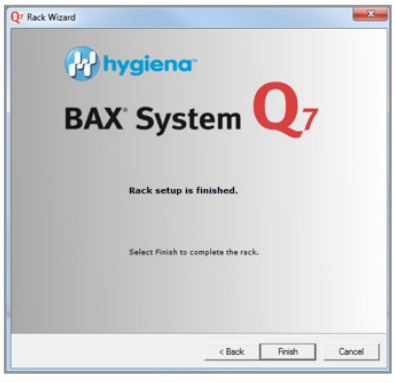


If you have created a new rack, a summary window appears that details the rack information. If you have imported a rack file, a shorter summary is displayed. After reviewing your definitions, click **NEXT** button to continue.



A final window appears that tells you the rack setup is complete.
Click **FINISH** to create a rack file with the name you assigned in the steps above and a ".bax" extension.

Note: *Once the rack file is created, you can change sample information by following the directions below for defining/editing wells.*



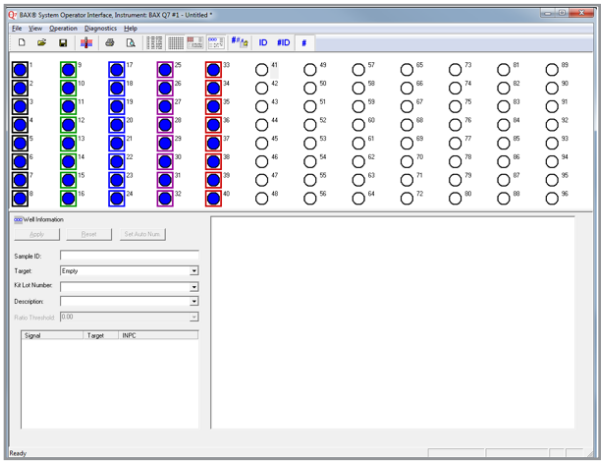
Manually Create a Rack File

1. At the empty rack view, select FILE > SAVE AS from the menu bar to create a new rack file with the ".bax" extension.

   Note: *If you don't save the file at this time, you are prompted to save it before the amplification begins.*

2. In the rack view lower pane, enter rack information:
   a. Enter a Label (optional) for the rack. Press the TAB key to move to the next field.
   b. Enter the Operator ID (optional).
   c. Enter a Description of this rack (optional).
3. Click on the **APPLY** button.

   Note: *After the rack file has been created, you can edit the information and click on the APPLY button to have those changes take effect on screen. However, the file itself is not modified until you select FILE > SAVE from the menu bar.*

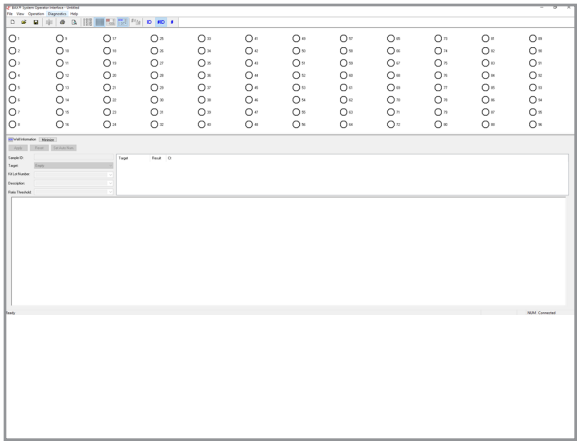


Defined rack

Define or Edit Wells

After you have defined your rack, you need to enter information on individual samples. This is done via the “well view” window, which appears when you click on a well in the upper pane. The upper pane of the window remains the same, but the lower pane changes to display information fields on each specific sample.

Switch to well view by clicking on a well in the upper pane. A different lower pane appears.



Empty well view

Note: *You can also get a well view by selecting VIEW > EDIT WELL INFO on the menu bar, or by clicking on the icon in the toolbar*

You can return to rack view any time by selecting VIEW > EDIT RACK INFO on the menu bar or by clicking on the icon in the toolbar

Enter Sample Information

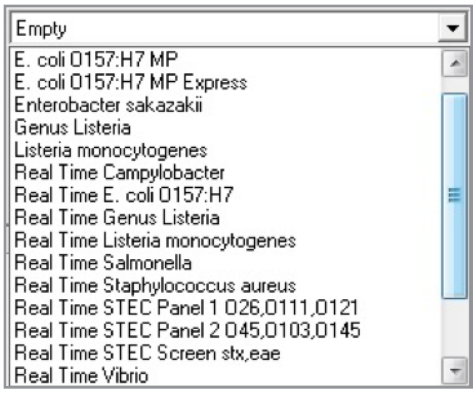
1. If you are not using the Auto Number feature (see next section), click on the first well, then enter the **Sample ID** (optional). Press the ENTER key to move to the next well and enter ID information.

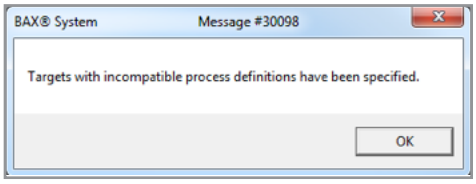
   Note: *If you press the TAB key, you will move to the next data field for that well. If you press the ENTER key at any time, you will move to the same data field for the next well.*

2. Select and highlight all the wells with the same target organism.

   Note: *You can enter data on a group of wells by clicking the mouse on the upper pane, and dragging it across a row or column to highlight the desired wells. You can also select a group by clicking on wells or columns, then pressing the CONTROL key and clicking on additional wells or columns. Any data you enter in the form is then applied to all the selected wells.*

3. Select the appropriate **Target** from the drop-down menu of possible targets. The **Control** type is automatically entered. These fields are required in order to run the process.





Note: *Some assays have multiple target choices. Refer to the assay instructions for use (product instructions) for guidance.*

Note: *Except for MP Express, and Yeast & Mold protocols, the standard BAX System PCR assays use the same set of cycling conditions, so you can test for mixed targets in a single rack. Some BAX System real-time PCR assays also share a common set of cycling conditions, allowing you to mix these assays in a single rack. However, you cannot combine standard assays and real-time assays in the same rack. If you try to select incompatible targets, an error message appears.*

Enter Sample Information (Continued)

4. Enter the BAX System Kit Lot Number from the box label (optional).
5. Use the Description field to enter any additional information (optional).
6. Click on the **APPLY** button. The grid displays blue filled circles to indicate defined samples. The list box in the lower half of the screen displays differently for standard PCR assays than for real-time PCR assays. Select FILE > SAVE from the menu bar.

Note: *You can edit information in this file prior to loading your samples into the instrument. After the samples have been processed, you cannot edit the file. Any changes must be saved under a new file name.*

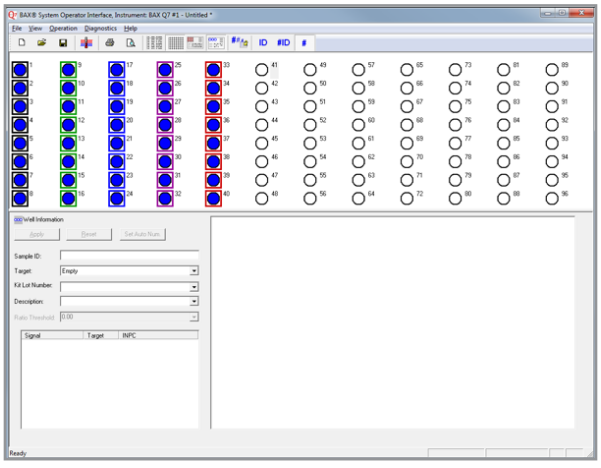
Setting Optional Preferences

Color Box Around Target Wells

Under the VIEW menu, you can select the COLOR TARGET WELLS toggle option. This will display a colored box around each well. Each color is specific to a target (also see kit label color), and thus provides a visual cue for tracking samples.

STANDARD ASSAYS	
Black:	*Salmonella*
Red:	*E. coli* O157:H7 MP *E. coli* O157:H7 MP Express
Blue:	*Listeria monocytogenes* (including 24E)
Green:	Genus *Listeria* (including 24E)
Mauve:	*Cronobacter*
Light Green:	Yeast and mold

REAL-TIME ASSAYS	
Black:	*Salmonella*
Orange:	*E. coli* O157:H7, HPS (*Salmonella*)
Red:	*E. coli* O157:H7 Exact, Exact + S
Orange + Yellow:	STEC Screening (stx and eae)
Orange + Brown:	STEC Panel 1 (O26, O111, O121)
Orange + Red:	STEC Panel 2 (O45, O103, O145)
Green:	Genus *Listeria*
Blue:	*L. monocytogenes*
Lilac:	*Staphylococcus aureus*
Aqua:	*Vibrio cholerae/parahaemolyticus/vulnificus*
Gold:	*Campylobacter jejuni/coli/lari*
Brown:	*Shigella*



Defined wells in a mixed batch with Color Target Wells selected

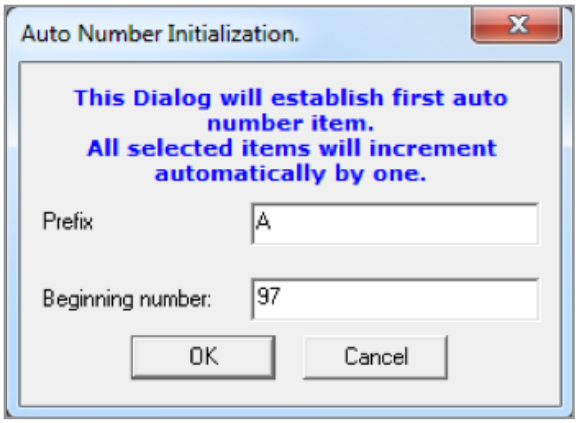
Auto-Number Wells

By default, the wells in the upper pane are numbered sequentially from 1 to 96. You can choose three different ways to display information in the upper pane by clicking on the appropriate icon in the tool bar:

#	Displays the well numbers only.
#ID	Displays the well numbers along with any Sample ID information you have entered in the lower pane.
ID	Displays only the Sample ID information you have entered for each well.

If desired, you can assign a different numbering scheme to your samples through the Auto Number feature. This option uses an alphanumeric sequence in place of Sample ID.

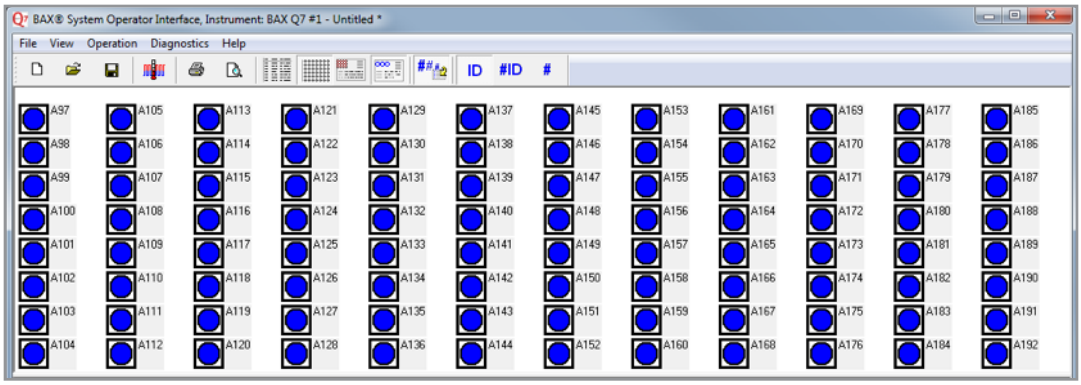
1. Make sure a Target (from the drop-down menu in the lower pane) has been selected for every well that contains a sample. When you click on **APPLY**, the selected wells change to blue-filled circles.
2. Select the group of wells you wish to auto-number.
3. Click on the ID icon in the tool bar, then click on the Auto Number icon. A window appears with two optional ID fields.



4. If desired, enter an alpha prefix (up to two characters) for all your samples.
5. If desired, enter a different initial number for this series of samples.
6. Click **OK**. The well view grid changes the labeling on blue-filled circles to reflect your auto-numbering scheme.

Note: *The auto-number scheme does not overwrite any Sample ID information that you have already entered manually.*

Auto-Number Wells (Continued)



Well view with Auto Number ON

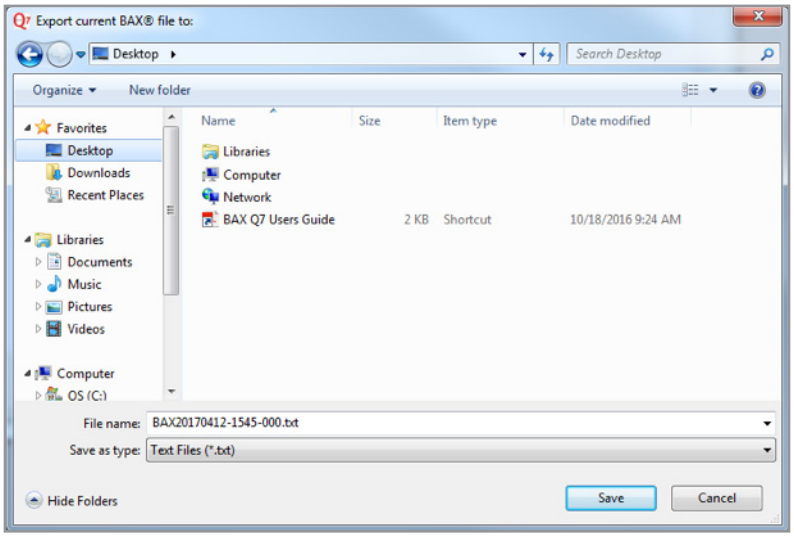
Note: *If desired, you can print a hard copy of these defined samples to use as a tracking sheet for the rest of the procedure. Click on either the* ***ID*** *or* ***#ID*** *icon, then choose FILE > PRINT > RACK REPORT from the menu bar.*

Smaller Well Icons

Under the VIEW menu, you can select the SMALLER WELL ICONS toggle option. This will re-size the display of the well icons to allow longer labels for each sample, both on-screen and in printed reports.

Configure Export Preferences

These options allow you to export information from your rack, including results, as tab-delimited text for integration with spreadsheets and other applications. You can export your rack file anytime by selecting FILE > EXPORT FILE from the menu bar. A “SAVE AS” export window appears that allows you to choose the folder in which to save this “.txt” file.

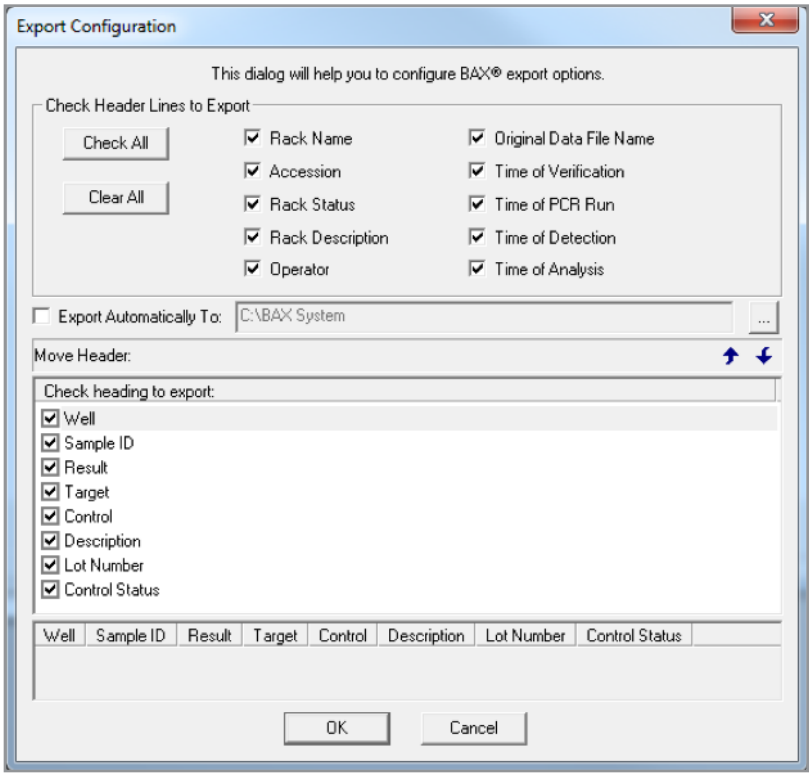


Export window

Configure Export Preferences (Continued)

You can configure your export preferences to include or exclude specific data fields, and you can re-order these fields to meet your import needs. You can also identify a folder to which this text file will automatically be exported at the end of the detection phase.

1. Under the FILE menu, select CONFIGURE EXPORT. A new window appears with three sections of options.
2. In the top section, check the data fields for rack and instrument information you wish to include in the header of the text file.
3. If you check the box in the center section, text files are automatically generated and exported at the end of the detection phase to the folder you select.
4. In the lower section, check the fields for sample information you wish to include in the text file. Click on one of the arrows in the “Move Header” box to move the highlighted field higher or lower in the order. A row of headings below these fields displays the order you have set for the columns.



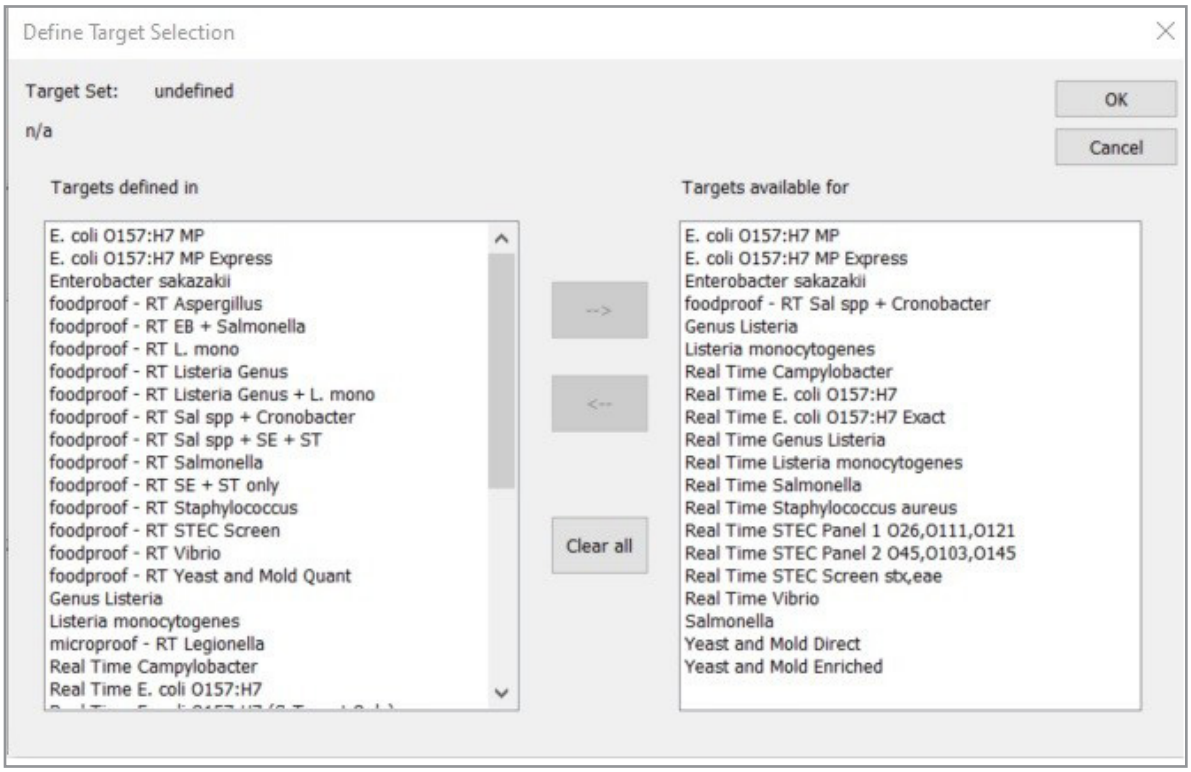
Export window

Select Targets

You can customize the list of targets in the drop-down Target menu (see “Window Sections” in Chapter VI: BAX System Software) to meet the needs of your lab.

Under the VIEW menu, choose the SELECT TARGETS option to display a dialogue box showing the complete system list of targets on the left and your defined list of targets on the right. To add to your defined list, highlight the desired targets in the system list, then click the right-arrow key. To remove targets from your defined list, highlight them, then click the left-arrow key. The CLEAR ALL button deletes all targets from the defined list.

Note: *For certain assays, you must manually add the target to your drop-down menu of available target programs.*



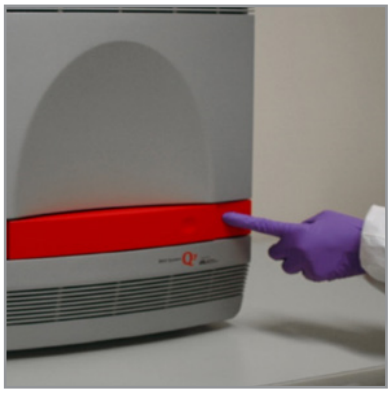
Define target selection dialogue box

Running a Full Process

Initialize the Instrument

Prior to loading samples for a full process run, the instrument requires about five minutes to heat to the appropriate temperature. We recommend that you initialize the instrument before hydrating tablets so that the program is ready to accept chilled samples immediately after you hydrate the PCR tablets.

1. Power on the BAX System Q7 instrument.

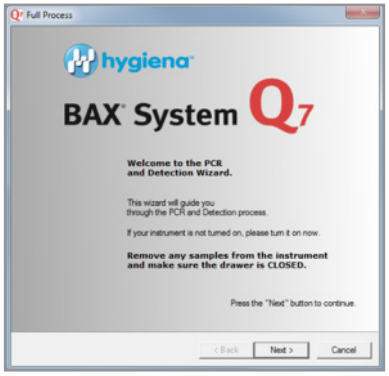


2. From the OPERATION menu, select RUN FULL PROCESS. This launches the PCR and Detection Wizard, which prompts you through the process.

   Note: *You can also launch a full process run by clicking the icon in the tool bar.*

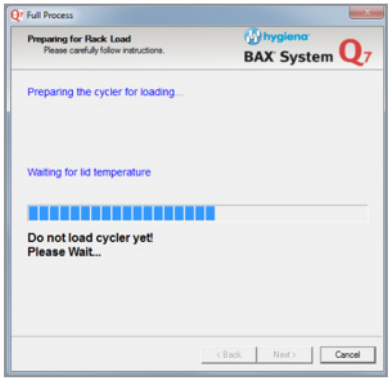
   

   If you have not saved your rack file, a navigation window appears for you to name and save the ".bax" file before processing.



A new screen automatically appears as the instrument begins to heat to its set temperatures. A message at the bottom of the screen warns you not to load samples yet.

While the instrument is heating, you can continue with lysis, if lysates are not already prepared.

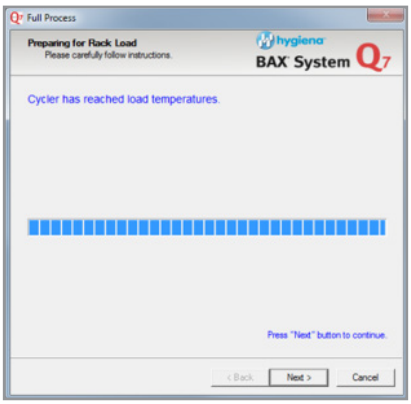


Amplify and Detect

PCR tablets must be loaded into the instrument within 30 minutes of hydrating in order to ensure accurate results. If you encounter a delay before loading, discard prepared samples and use additional sample lysate from cluster tubes to hydrate new PCR tablets as described above.

Follow the screen prompts in the PCR Wizard to load your samples, run the program and unload your samples:

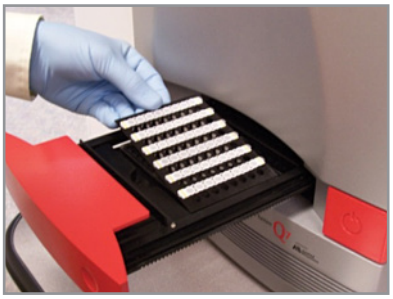
Preparing for Rack Load – displayed while the instrument warms to the correct temperature for loading the rack. Flashing text prompts you to wait. When the instrument reaches the appropriate load temperature, the wizard prompts you to click the NEXT button to continue.

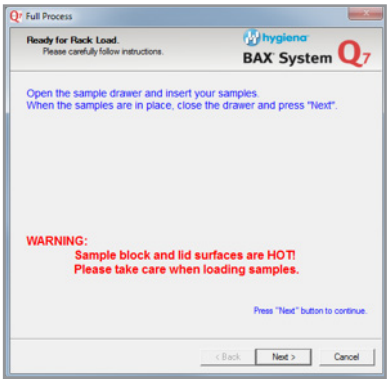


Ready for Rack Load – prompts you to load your samples.

1. Open the drawer by pressing the dimpled area on the right side.
2. Place the rack of PCR tubes into the instrument according to your rack file. Visually check to make sure the tubes are seated correctly and that the caps are sealed tightly.
3. Press the dimpled area to the right and push gently to close the drawer.
4. Click the NEXT button.

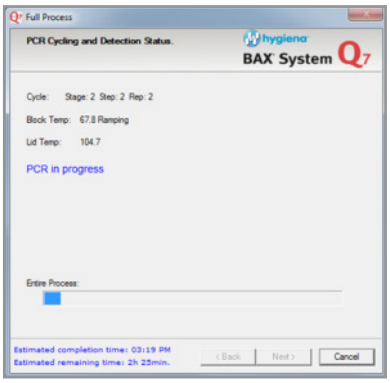
CAUTION: The heating block below the drawer is HOT! Take care to avoid touching this heated surface.





Amplify and Detect (Continued)

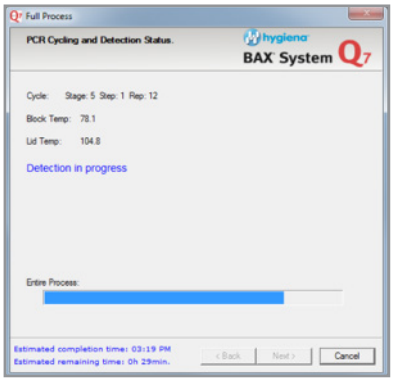
PCR Cycling Status –displays a status bar while the amplification portion of the program is running



Detection Status – displays a status bar while the detection portion of the program is running.

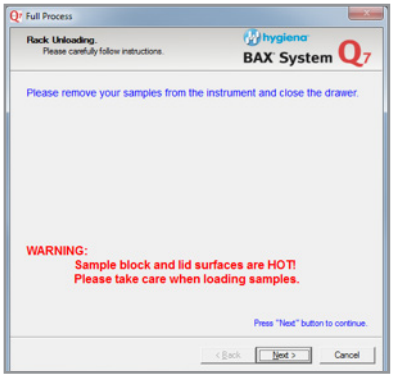
When this phase is finished, the instrument automatically shuts off heat to the block. If you have selected the automatic export option, a text file is sent immediately to the designated folder.

Note: *This screen does not appear when running real-time PCR assays because detection is integrated with amplification.*



Rack Unloading – prompts you to remove your samples. Open the drawer and carefully remove the rack of PCR tubes. Place the rack of samples in a bag and store in the refrigerator until you have finished reviewing the results, then dispose of waste according to your site practices.

Racks should be wiped with 10% bleach, rinsed with deionized water and blotted dry.



Process Completed Normally – Click the **FINISH** button to review the results on your processed samples.

Processing time

For standard PCR assays, automated amplification and detection in the BAX System instrument takes approximately 3.5 hours to complete. The *E. coli* O157:H7 MP Express protocol reduces the processing time to approximately 2.5 hours.

For BAX System real-time PCR assays, processing is complete in 65 to 90 minutes, depending on the assay; some foodproof assays take longer (see processing times in chart below).

Some BAX System assays can be run together in a single process in the BAX System instrument. Refer to the table below to determine which assays are compatible.

STANDARD PCR ASSAYS		REAL-TIME PCR ASSAYS	
Compatible Assays	**Processing Time**	**Compatible Assays**	**Processing Time**
BAX PCR assay for *Salmonella* 2 BAX PCR assay for *E. coli* O157:H7 MP BAX PCR assay for *Cronobacter* BAX PCR assay for Genus *Listeria* BAX PCR assay for *L. monocytogenes* BAX PCR assay for Genus *Listeria* 24E BAX PCR assay for *L. monocytogenes* 24E	3 h, 30 minutes	BAX Real-time PCR assay for *Salmonella* BAX Real-time PCR assay for Genus *Listeria* BAX Real-time PCR assay for *L. monocytogenes*	75 minutes
		BAX Real-time PCR assay for *Campylobacter* BAX Real-time PCR assay for *Staphylococcus aureus* BAX Real-time PCR assay for *Vibrio*	70 minutes
		BAX Real-time PCR assay for *E. coli* O157:H7 BAX Real-time PCR assay for *E. coli* O157:H7 Exact BAX Real-time PCR assay for STEC Suite BAX Real-time PCR assay for *Shigella* BAX Real-time PCR assay for *E. coli* O157:H7 Exact + S	65 minutes
		foodproof® Real-time PCR assay for *Staphylococcus*	72 minutes
		foodproof Real-time PCR assay for *L. monocytogenes* foodproof Real-time PCR assay for *Listeria* Genus	86 minutes
BAX PCR assay for *E. coli* O157:H7 MP Express	2 h, 30 minutes	foodproof Real-time PCR assay for *Salmonella* foodproof Real-time PCR assay for *Salmonella* + *Cronobacter* foodproof Real-time PCR assay for *Salmonella* spp + *S.* Enteritidis (SE) + *S.* Typhimurium (ST) foodproof Real-time PCR assay for *Listeria* Genus + *L. monocytogenes* foodproof Real-time PCR assay for STEC Screen foodproof Real-time PCR assay for *Vibrio*	84 min
		BAX® System Real-Time PCR Assay Highly Pathogenic ***Salmonella***	84 min
BAX PCR Assay for Yeast and Mold	3 h, 45 minutes	foodproof Real-time PCR assay for EB + *Salmonella*	103 minutes
		foodproof Real-time PCR assay for *Aspergillus*	115 minutes
		foodproof Real-time PCR assay for *Legionella*	2 h, 21 min
		foodproof Real-time PCR assay for Yeast & Mold, Quant	2 h, 24 min

Reviewing Results

Standard & Real-Time PCR Assays

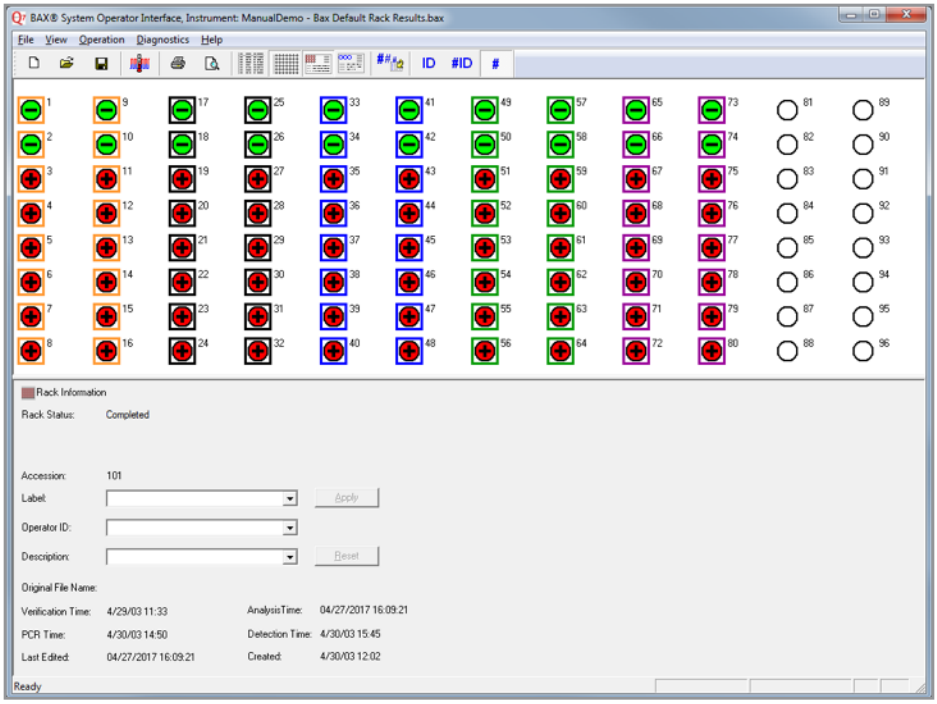
After you click on the **FINISH** button, a new window displays a modified rack view (see next page). The wells now appear in different colors with a symbol in the center to illustrate the results. You can view these results in rack view, rack details, well view and well details, as shown below.

	Green (-)	=	Negative result
	Red (+)	=	Positive result
	Yellow	=	Indeterminate result (see Appendix D for troubleshooting)
	Yellow (?) with red slash	=	Signal error (see Appendix D for troubleshooting)
	Orange with check mark	=	• Yeast & Mold Direct test has run successfully – see Detail View for ratio value • foodproof® assay - indicates assay is not using a certified or experimental algorithm • In HPS - indicates a second enrichment step is needed

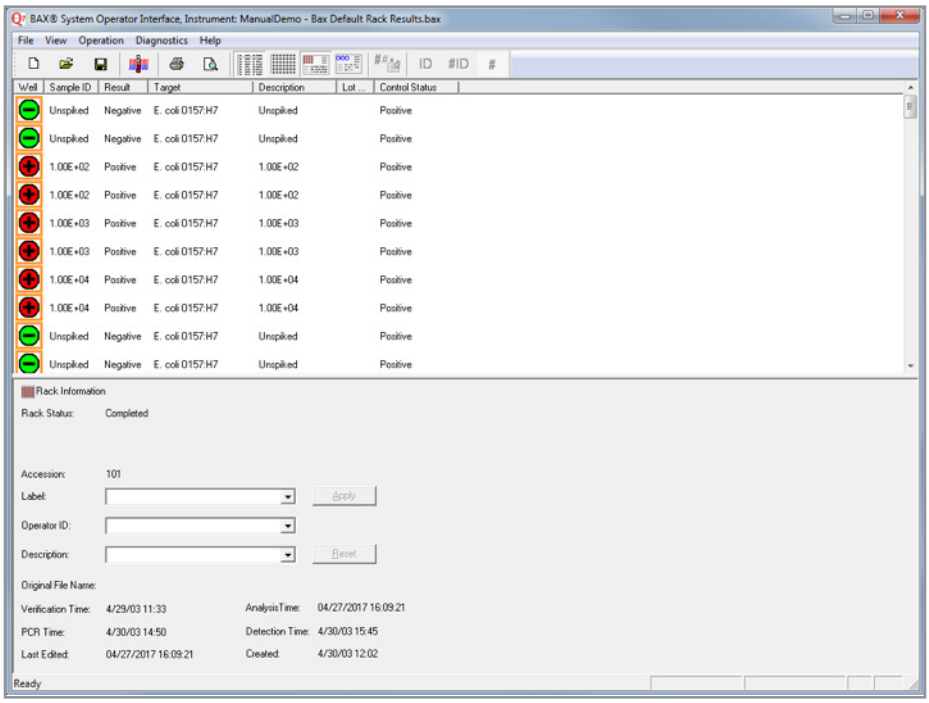
Note: Additional symbols may be displayed for specific assays, please refer to the kit product instructions (IFU) for complete details.

Rack View

The rack view shows all results along with information on the rack. You can display the rack results as a grid or as a detailed list.



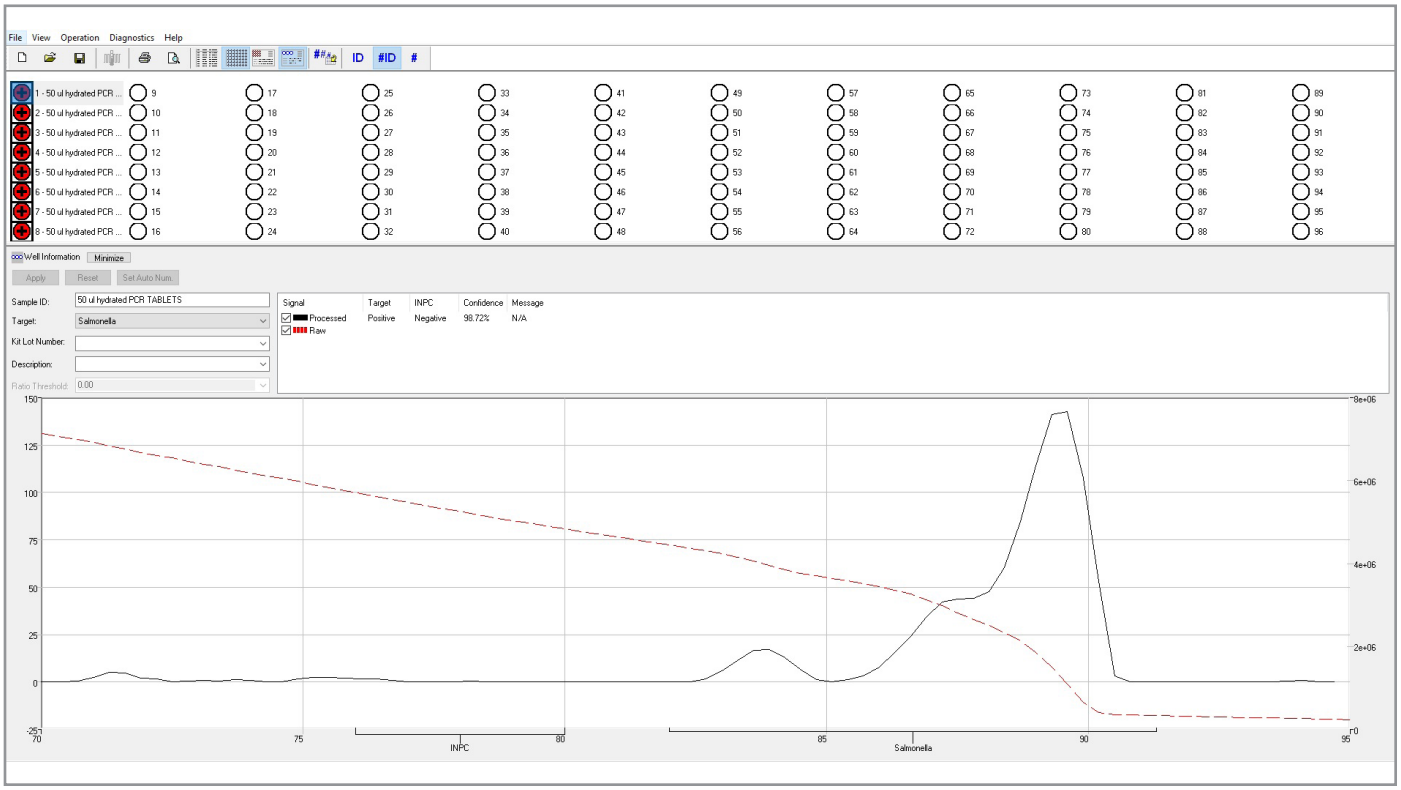
Results in rack view



Results in rack detail view

Well View

You can examine the information on specific wells (or groups of wells) by selecting them. The list box in the lower pane then displays processed data with results for target and positive control in the list box. In standard PCR assays, the melting curve (solid black line) for processed data displays in the graph to the right. You can choose to overlay the raw data curve (red dashed line), if desired, by clicking on that option in the list box. You can also choose to display the upper pane as a detailed list.

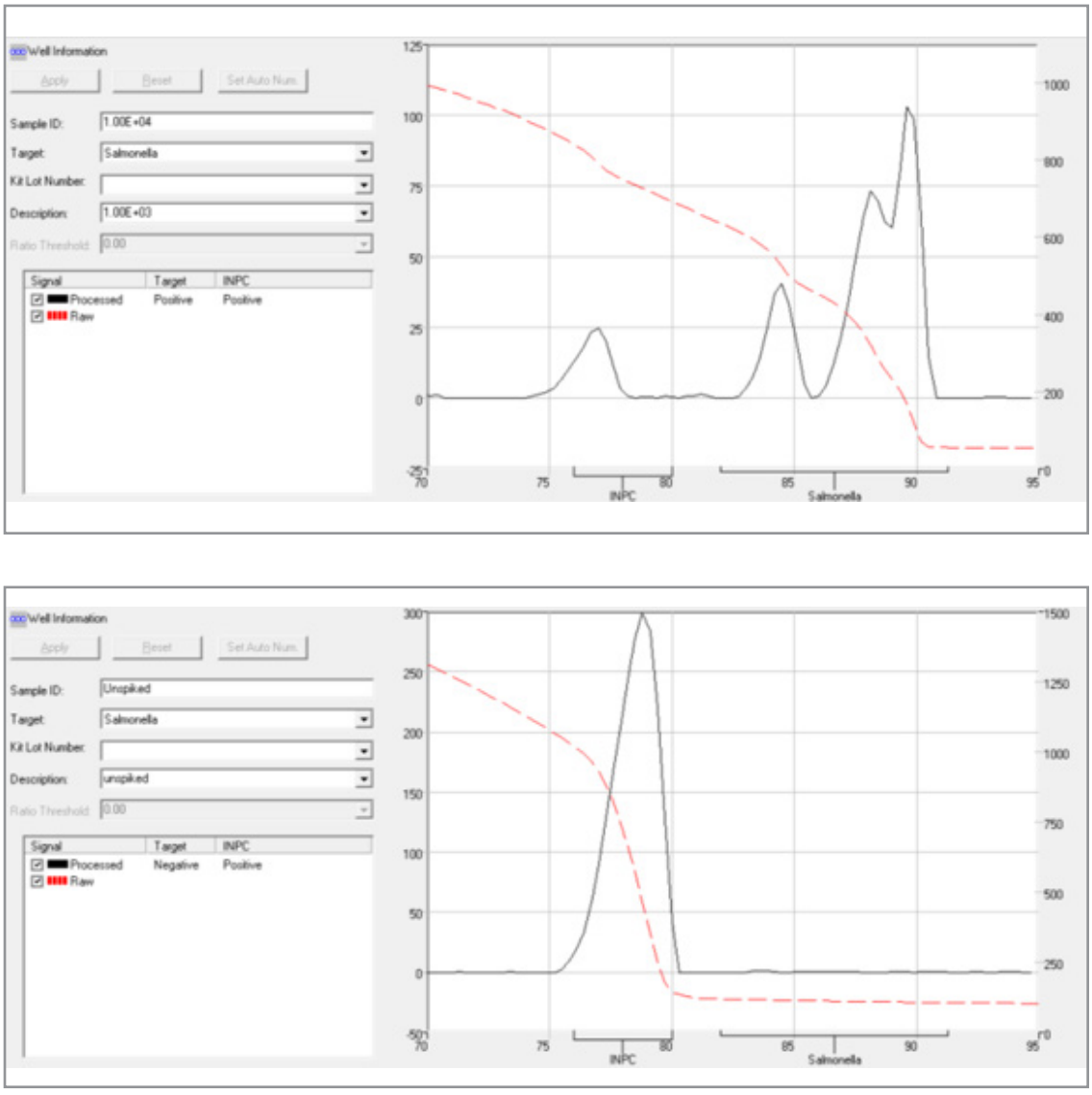


Results in well view

Melting Curves for Standard Assays

The melting curves produced by each target organism vary in the number and location of target peaks. See the melting curve profiles at the end of each BAX System assay protocol for details.

Note that the lower pane of the well view contains a line graph of processed and/or raw data associated with the selected wells. The processed data graphs allow you to view the melting curve, which is unique to each target. The raw data displays the unprocessed fluorescent intensity against temperature.

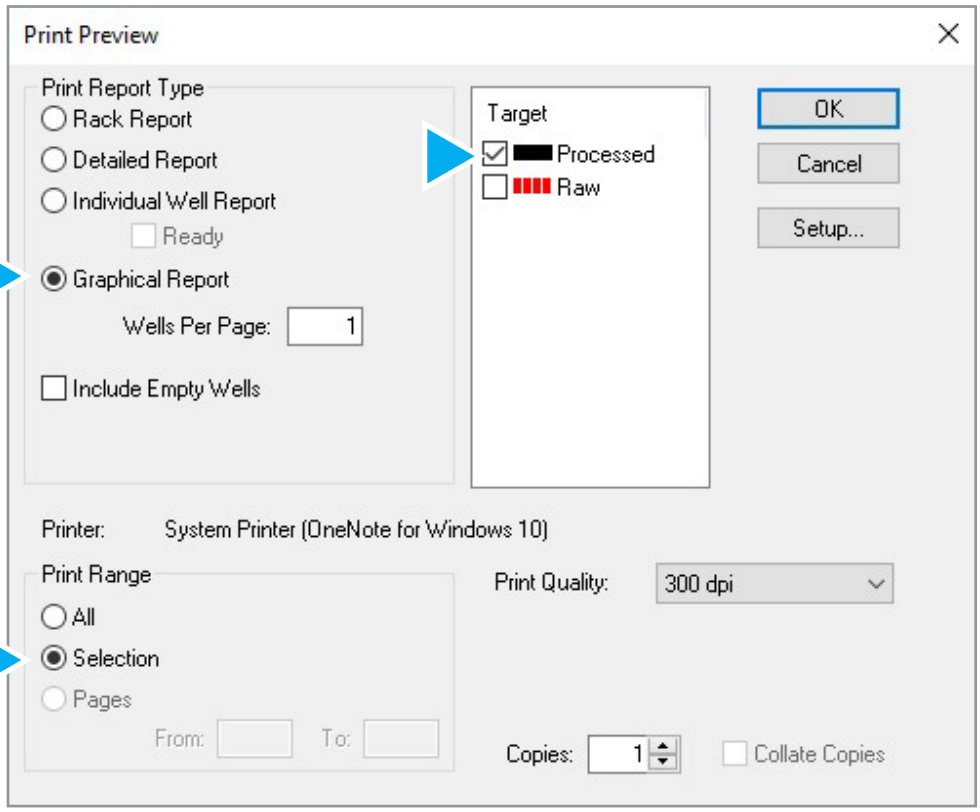


Typical positive (top) and negative (bottom) melting curve profiles

Typical Melting Curve Profiles

The BAX System application provides several graphs of data for each reaction. You can examine a Processed view of the melting curve and interpret the graph as follows:

1. In the BAX System results window, click on the well you want to review.
2. To enlarge the graph for easier viewing, select FILE > PRINT PREVIEW. A print options window appears.
3. Click on Graphical Report (1 Well Per Page), Processed View and Selection. If you have selected more than one well, the Selection option is automatically activated.



4. Zoom to enlarge the graph for viewing by clicking on the graph with the zoom tool. You can click the zoom tool two times.

For All Standard Targets

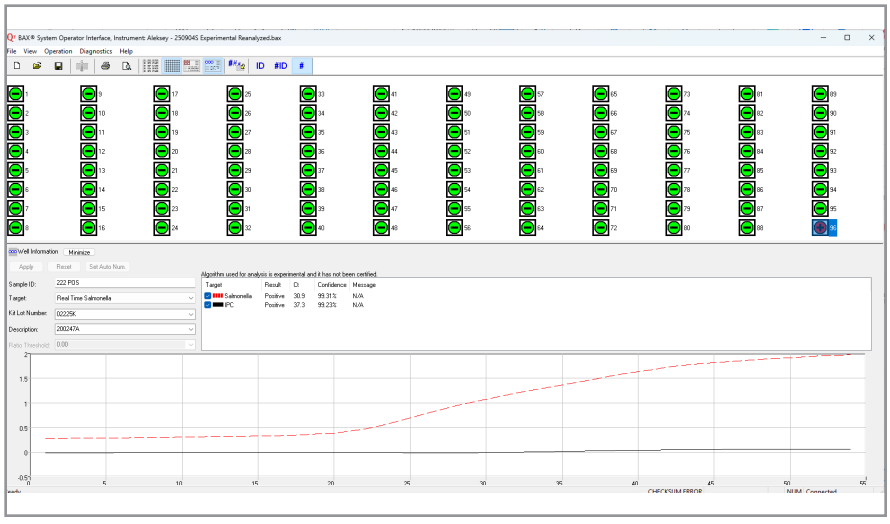
Target peaks - positive reactions show target peaks within a specified temperature range. Peaks can shift within their temperature range. In strong positive reactions, the peaks can shift to slightly lower temperatures, and in weaker reactions, the positives can shift to higher temperatures. Only peaks in the expected locations should be used for interpretation. See the melting curve profiles in each IFU for the BAX System assay protocol you are using.

Control (INPC) peaks - occur in a specific temperature range for each target. A control peak should be visible at approximately 79 °C for negative reactions. See the melting curve profiles in each IFU for the BAX System assay protocol you are using. Control 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.

Amp Plots for Real-Time Assays

The results of real-time assays are displayed differently from the results of standard assays. Because real-time PCR assays use probe-based chemistry instead of fluorescent dye, the system does not generate melting curves. Instead, the graph on the right displays amplification plots for each target. For more details on typical amp plots for each real-time target, see the amplification plot profiles in each product instruction protocol for each individual BAX System assay.



Results for real-time assays

For All Real-Time Targets

Fluorescence Intensity Units (FIU) – the Y-axis of the amp plot represents the level of fluorescence intensity units (FIU) measured by the BAX System instrument. The X-axis represents the number of heating and cooling cycles the BAX System performed.

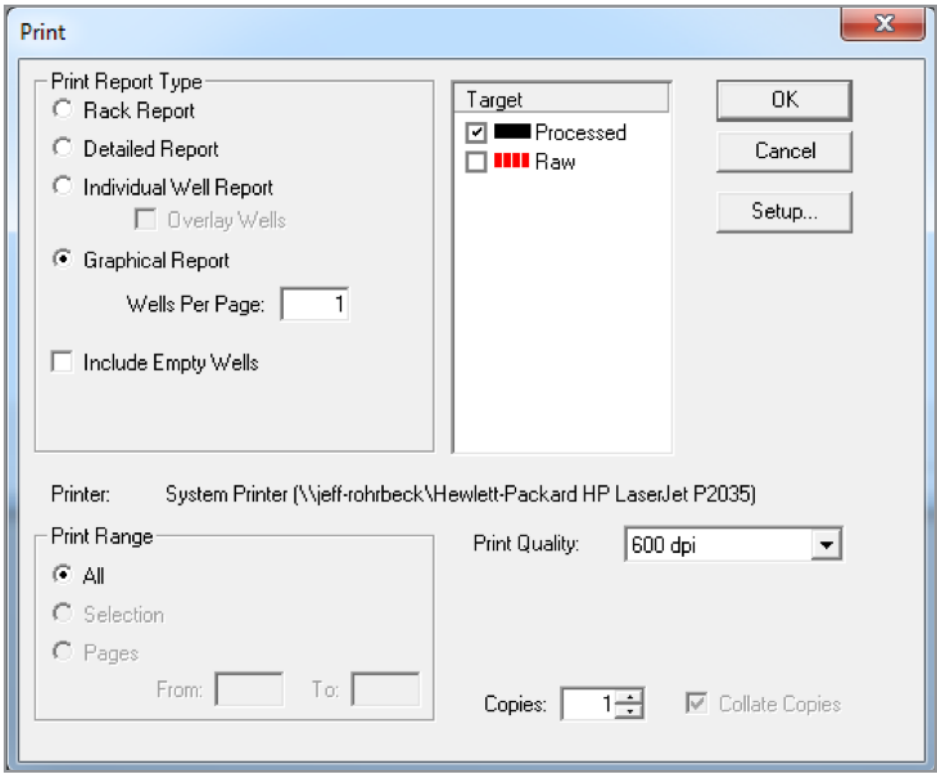
Sigmoid-shaped curves – positive reactions appear on the amp plot as a sigmoid-shaped curve. For a reaction to be considered positive, the Ct (or threshold crossing) value of this curve must be a positive value, typically this value will be between 20 and 50. A lower Ct value usually indicates a higher concentration of the target in a sample.

Internal Positive Control (IPC) – each amp plot displays an additional black curve that serves as in internal positive control. This INPC curve usually has a positive Ct value. The Ct value of the IPC curve is generally between 30 and 40. If both the INPC reaction and the target reaction are negative, an indeterminate result will be displayed. (Note: the INPC can be negative as long as the targets are positive.)

For more information on typical amp plots, see the amplification plot profiles in each product instruction protocol for each individual BAX System assay.

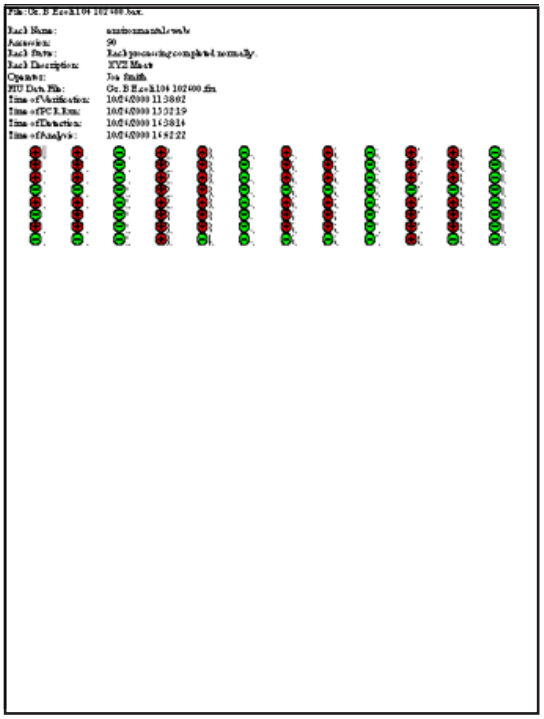
Printing Reports

You can print several types of reports by selecting FILE > PRINT PREVIEW from the menu bar and clicking on the appropriate option. You can designate the type of report for all or selected samples, along with the data type as individual or overlaid graphs.

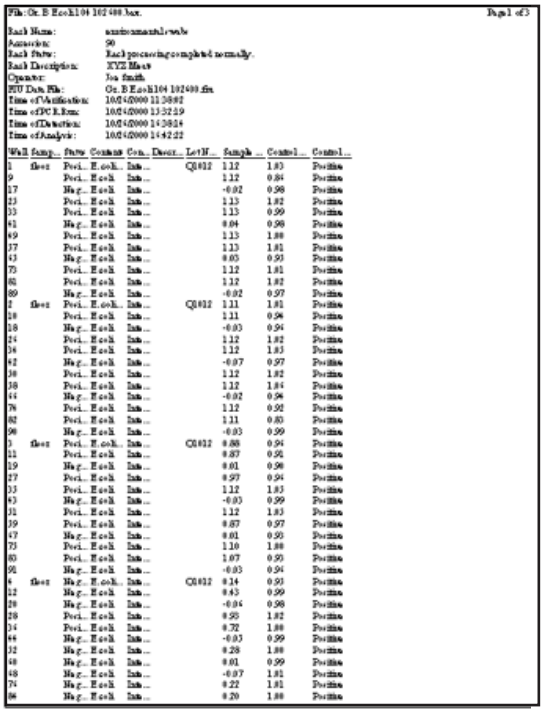


Print options

Every report includes the name of the file, the application version, and the instrument name on which the samples were processed.

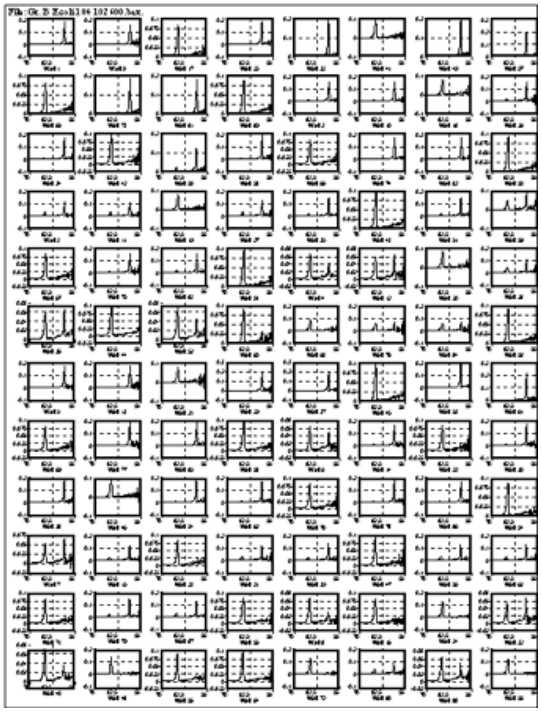


Rack Report: Provides a single-page print-out summarizing the results of all 96 wells as displayed in the Well Grid View.

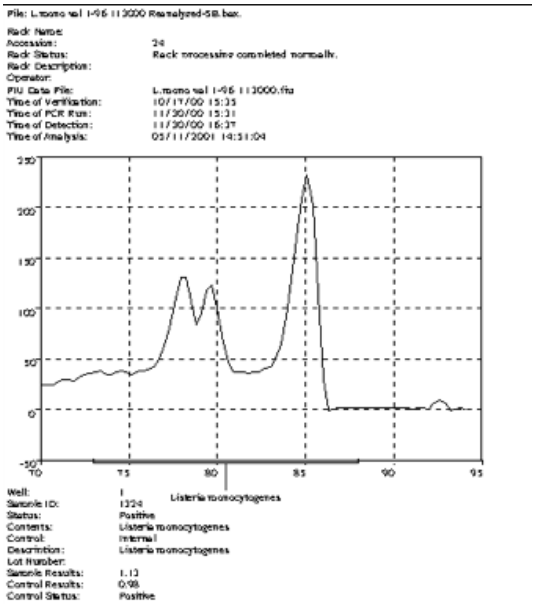


Detail Report: Provides a single-page print-out summarizing the details of each well result as displayed in the Well Detail View.

Note: *If the complete details for each column do not appear in the report, select the "Details View" on the main screen, then click and drag the edges of the column headers to expand each column before printing.*



Graphical Report: Provides a melt curve image for each sample selected (up to 96 samples) as displayed in the Well View.



Individual Well Report: Provides a detailed report for each sample selected (up to 96 samples), including the melt curve image as displayed in the Well Detail View.

Upgrading the Software

Upgraded versions of the BAX System software are released regularly to include new assays, enhanced algorithms and other improvements. Using the most current software release is always recommended with the BAX System. Follow these instructions to install an upgraded version of the BAX System software onto the computer.

1. Back Up the Calibration File

Prior to upgrading or reinstalling the BAX System application, you must back up the system's calibration file using the EXPORT CALIBRATION command. This safeguards against the file being lost or overwritten during reinstallation of the application.

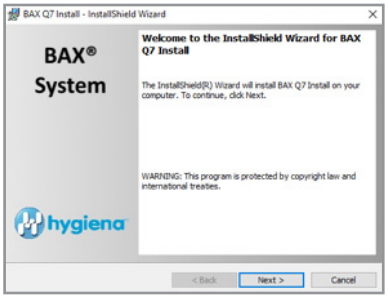
1. From the OPERATIONS menu, select BAX MAINTENANCE > EXPORT CALIBRATION, then navigate to a new folder in which to store the backup file. Name the calibration file (example: "Q7Cal_date.bqc").
2. Click **SAVE** .

2. Install/Reinstall the Application

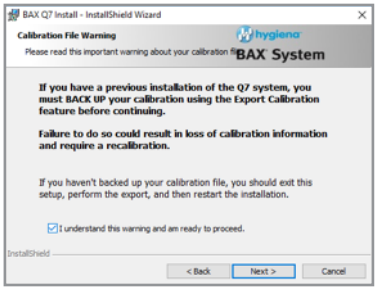
After backing up your calibration file, you are ready to install a new release or re-install another version. Be sure to follow the instructions for the version of software you are installing. Before proceeding with the installation, the previous version needs to be uninstalled first. Be sure the Regional and Language settings are set to English (United States) and English, respectively.

1. Close all programs and files. Download the proper software from www.Hygiena.com, copy it to your desktop, and open the .exe file. Click **INSTALL**.

2. When the install wizard appears, click **NEXT**.

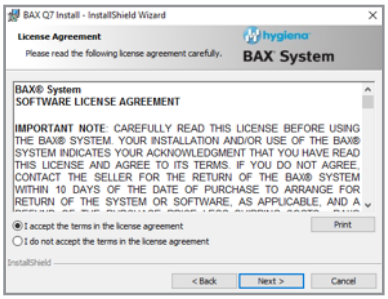


3. A screen appears with a reminder to backup previous calibration files. If you have not backed up these files, click **CANCEL** and perform the export. If these files have been exported, click **NEXT**.

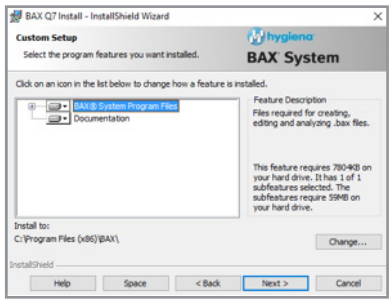


2. Install/Reinstall the Application (Continued)

4. The EULA screen appears. Select the “I accept…” option and click **NEXT** to accept the terms of the EULA.

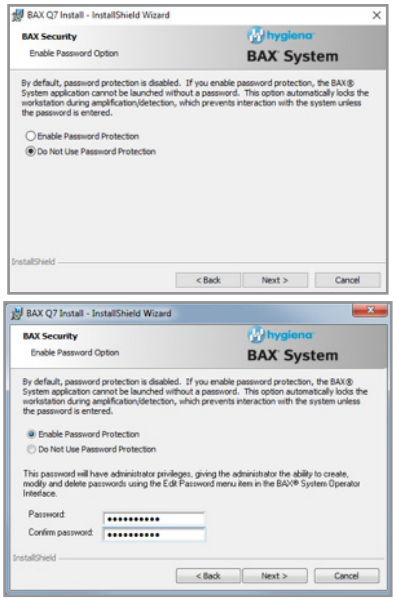


5. A window appears in which the features to be installed are pre-selected. Click **NEXT** to continue.



6. A message box displaying the password option appears. To skip password protection, select the “Do not use password protection” option, then click **NEXT** .

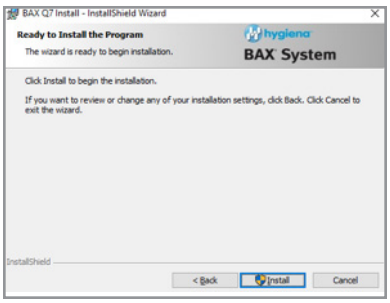
   To enable password protection, select the “Enable password protection” option. Enter a password for the BAX System application, and click **NEXT** .

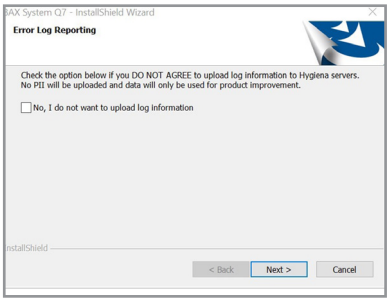


2. Install/Reinstall the Application (Continued)

7. The New Installation window appears. Click **INSTALL** to begin the installation.

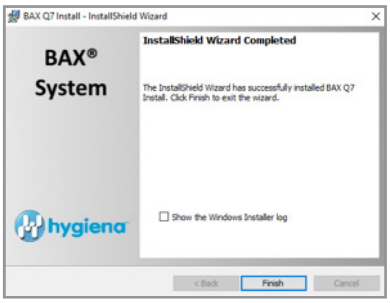
   A message box displaying the upload error messages option appears. Select the option "Upload error messages" or to skip this option, select "Do not upload error messages" option, then click **NEXT**.





8. Follow the wizard prompts to finish installation. You do not have to restart your computer for the security features to take effect.

9. Shut down the BAX System instrument and computer, and then restart the system. Double-click the desktop icon to launch the updated software

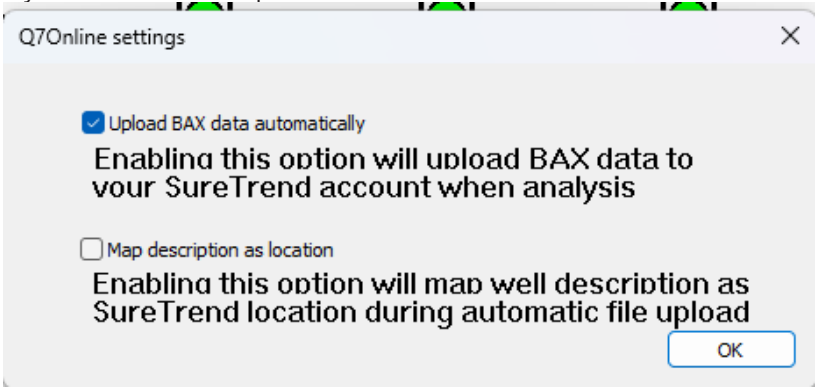


3. Check the Calibration File

After installation, the calibration files should be installed automatically. To check that these files are installed successfully, select DIAGNOSTICS > CALIBRATION and click the Check Calibration Box in the BAX System menu bar to open the Q7 Calibration Diagnostics window and determine the status of your calibration files. If calibration coefficients are missing, import the backed-up calibration file by selecting OPERATIONS > BAX MAINTENANCE > IMPORT CALIBRATION from the menu bar and selecting the backed-up calibration file.

Backing Up Data

For an automated, encrypted, and reliable backup, connect you BAX Q7 to STC and enable automatic data sync. This will allow your entire team to securely access BAX Q7 results and collaborate 24/7 from anywhere. Under Operation->BAX Maintenance->Q7 Online enable automated BAX data upload:



Alternatively, if you can backup up your data manually. We recommend that you back up your data frequently (at least once a week). Your BAX System startup package includes a removable storage device. Please see the computer manufacturer's documentation for instructions on backing up data.

Appendix A. Enrichment Media Recipes

Appendix A. Enrichment Media Recipes

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

Reference method source	Link
USDA-FSIS Microbiology Laboratory Guidebook	https://www.fsis.usda.gov/sites/default/files/media_file/documents/MLG-Appendix-1.14.pdf
FDA Bacteriological Analytical Manual	https://www.fda.gov/food/laboratory-methods-food/media-index-bam
Health Canada Compendium of Analytical Methods	https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods/official-methods-microbiological-analysis-foods-compendium-analytical-methods.html

Important Tips on Enrichment Media

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

24 LEB Media (for some *Listeria* Assays)

Using 24 LEB Complete (blended media)

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

24 LEB Media with Buffer Supplement (for 24E Assays)

Required by NF VALIDATION for smoked fish with 24E assays and charcuteries with *L. monocytogenes* 24E assay

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

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

Alkaline Peptone Water

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Peptone	10 g	Dissolve peptone and NaCl into distilled water. Adjust pH so that value after sterilization is 8.5 ± 0.2. Dispense into screw-cap tubes. Autoclave at 121 °C for 10 minutes.
NaCl	10 g	
Distilled water	1 L	

Note: *Commercial preparations must be diluted to the concentration above before use with the Vibrio assay. If using Oxoid CM1117 dehydrated Alkaline Saline Peptone Water (www.oxoid.com), 20 g base powder should be rehydrated with 1 L of water instead of following the directions on the package in order to reach the correct concentration.*

BAX System MP Media

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

StatMedia™ soluble packets

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

Bulk powder

- BAX System MP media – Catalog No. MED2003 – 2.5 kg
- Dissolve 22.5 g in 1 L deionized water. Adjust pH to 7.2 ± 2 at 25 °C, then autoclave at 121 °C for 15 minutes.

BAX System *Listeria* Broth

- BAX System media for *Listeria* – Catalog No. MED2002 – 2.5 kg
- Dissolve 60.4 g in 1 L deionized water. Adjust pH to 7.2 ± 2 at 25 °C, then autoclave at 121 °C for 15 minutes.

  CAUTION: Do not boil the media or overheat the media in the autoclave. Do not extend autoclaving beyond 15 minutes.

Bolton Broth with Supplement (Single Strength)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Meat peptone	10 g	**THOROUGHLY MIX** 27.61 g in 1 L deionized water. Let soak 10 minutes. Swirl again and autoclave at 121 °C for 15 minutes (in screw-capped bottles if possible). Before use, add 2 rehydrated vials of *Campylobacter* enrichment broth (Bolton formula) supplement (Oxoid SR0183 or Malthus Diagnostics X-131). If supplement is not available add 4 mL each of antibiotic concentrates (See http://www.cfsan.fda.gov/~ebam/m28a.html). Final pH, 7.4 ± 0.2. Do not add blood to this mix. Store powdered media in a tightly fastened container in a cool, dry area to reduce oxygen infusion and peroxide formation, which can inhibit recovery of microaerophiles. Use prepared broth within 1 month of preparation (preferably less than 2 weeks).
Lactalbumin Hydrolysate	5 g	
Yeast extract	5 g	
NaCl	5 g	
Haemin	0.01 g	
Sodium pyruvate	0.5 g	
Alpha-Ketoglutamic acid	1 g	
Sodium metabisulphite	0.5 g	
Sodium Carbonate	0.6 g	
Deionized water	1 L	

Note: *Commercial preparations are available from Oxoid (www.oxoid.com) for Bolton broth base (CM0983) and supplement (SR0183).*

Bolton Broth with Supplement (Double Strength)

Prepare as above but use 55.22 g dry ingredients and add 4 vials of reconstituted supplement.

Brain Heart Infusion (BHI) Broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Calf brain, infusion from, 200 g	7.7 g	Dissolve ingredients in deionized water with gentle heat. Autoclave at 121 °C for 15 minutes.
Beef heart, infusion from, 250 g	9.8 g	
Proteose peptone (Difco) or polypeptone (Bioquest)	10 g	
NaCl	5 g	
Na_2HPO_4	2.5 g	
Dextrose	2.0 g	
Deionized water	1 L	

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

Brain Heart Infusion (BHI) Broth – Double Strength with NaCl

Dissolve 74 g of BHI base and 140 g of NaCl in 1 L of deionized water, and mix. Final pH, 7.4. Autoclave at 121 °C for 15 minutes, and cool to 37 °C before use.

Brilliant Green Dye Solution (1%)

Source: U.S. FDA Bacteriological Analytical Manual

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

Buffered *Listeria* Enrichment Broth (BLEB)

Source: U.S. FDA Bacteriological Analytical Manual

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

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

Buffered Peptone Water

Source: U.S. FDA Bacteriological Analytical Manual

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

Note: *Commercial preparations are available from Hygiena (Part No. MED2010) 2.5 Kg, or (Part No. MED2011) 500 g, or from Oxoid (#CM0509).*

Buffered Peptone Water with Novobiocin

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

Butterfield's Phosphate-Buffered Dilution Water

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
KH_2PO_4	34 g	Dissolve in 500 mL deionized water. Adjust pH to 7.2 with 1 N NaOH. Bring volume to 1 L with additional deionized water. Sterilize at 121 °C for 15 minutes. Store in refrigerator.
Deionized water	500 mL	

Complete Selective Enrichment Broth

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

Dey-Engley (D/E) Neutralizing Broth

Source: U.S. FDA Bacteriological Analytical Manual

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

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

Demi-Fraser Broth

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

Note: *Ferric ammonium citrate is not required in this formulation.*
Commercially available preparations are also available from Oxoid as Fraser broth base (#CM0895) and Half-Fraser Supplement (#SR0166).

EHEC Enrichment Broth (EEB)

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Dissolve ingredients without antibiotics in deionized water. Autoclave at 121 °C for 15 minutes. Cool to room temperature before adding the filter-sterilized antibiotics. Final pH, 7.4 ± 0.2. ***Note:*** *The level of cefixime has been reduced to ¼ strength from that described in BAM, Edition 8, Revision A /1998, because the original concentration of 0.05 mg/L was found to be inhibitory to the growth of O157:H7 positive control strains in the absence of competing microflora.*
Bile salts #3	1.5 g	
Dipostassium phosphate (K_2HPO_4)	1.5 g	
Deionized water	1 L	
Cefixime	0.0125 mg	
Cefsulodin	10 mg	
Vancomycin	8 mg	

Giolitti-Cantoni with Tween and Tellurite (GCTT) broth

Ingredients	Amount	Instructions
Giolitti-Cantoni broth (Oxoid CM0523 or equivalent)	54.2 g	Dissolve Giolitti-Cantoni broth in deionized water. Add 1 g Tween 80 to dissolved broth and mix. Final pH, 6.9 ± 2. Autoclave at 121 °C for 15 minutes, and cool to 37 °C. Just before use, add 15.8 mL sterile solution of 3.5% Potassium tellurite per L.
0.1% Tween 80	1 g	
3.5% Potassium tellurite	15.8 mL	
Deionized water	1 L	

Lactose Broth

Source: U.S. FDA Bacteriological Analytical Manual

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

Modified EC broth with Novobiocin (mEC+n)

Ingredients	Amount	Instructions
Tryptone	20 g	Dissolve ingredients in deionized water. If necessary, adjust pH to 6.9 ± 0.1 with 1 N HCl before autoclaving. Autoclave at 121 °C for 15 minutes and cool. Add 5 mL of a filter sterilized, aqueous solution of 4mg/mL sodium novobiocin (adjusted for potency; Sigma N1628) for each L of medium (20 mg/L). Final pH, 6.9 ± 0.1 at 25 °C.
Bile Salts #3	1.12 g	
Lactose	5 g	
K_2HPO_4	4 g	
KH_2PO_4	1.5 g	
NaCl	5 g	
Deionized water	1 L	
Novobiocin	20 mg	

Modified LST Broth with Vancomycin

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Tryptose or trypticase	20 g	Dissolve ingredients without antibiotic in deionized water. Autoclave at 121 °C for 15 minutes. Prepare vancomycin as a 10 mg/mL solution in deionized water. Filter sterilize through a 0.2 µm filter, then transfer 1 mL vancomycin solution to 1 L of the cooled (< 50 °C) mLST broth. Final pH, 6.8 ± 0.2. Use broth within 24 hours of adding the antibiotic. ***Note:*** *You can also use commercial brands of LST media, but you need to add extra NaCl (29.22 g) to the broth before autoclaving*
Lactose	5 g	
K_2HPO4	2.75 g	
KH_2PO_4	2.75 g	
NaCl	34.22 g	
Sodium lauryl sulfate	0.1 g	
Deionized water	1 L	
Vancomycin	10 mg	

Modified TSB with Novobiocin (mTSB+n) – FDA

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Dissolve ingredients without antibiotic in deionized water. Autoclave at 121 °C for 15 minutes and let cool to room temperature. Final pH, 7.4 ±.2. Add novobiocin just before use.
Bile salts #3	1.5 g	
Dipostassium phosphate	1.5 g	
Deionized water	1 L	
Novobiocin solution (R50)	0.2 mL	

Modified TSB with 8 mg/L Sodium Novobiocin (mTSB+n) – USDA

Source: USDA-FSIS Microbiology Laboratory Guidebook

Ingredients	Amount	Instructions
Modified Trypticase soy broth (mTSB) (Oxoid CM0989B or equivalent)	33 g	Dissolve ingredients without antibiotic in deionized water. Autoclave at 121 °C for 20 minutes. Let media cool to approximately 50 °C. Add 2 mL filter-sterilized, aqueous solution of 4 mg/mL sodium novobiocin for each L of medium. Final pH, 7.4 ± 0.2 at 25 °C. If refrigerated, media must be pre-warmed to 18-35 °C before use.
Casamino acids (casein acid hydrolysate)	10 g	
Deionized water	1 L	
Sodium novobiocin solution (4 mg/mL concentration) (Sigma N1628 or equivalent)	2 mL	

MOPS-BLEB* Broth

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

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

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

Nonfat Dry Milk, Reconstituted

Source: U.S. FDA Bacteriological Analytical Manual

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

Shigella Broth with Novobiocin

Source: China GB 4789.5-2012

Ingredients	Amount	Instructions
Shigella broth (BD #214915 or equivalent)	500 g	Prepare the *Shigella* broth according to the manufacturer's instructions. Dissolve 50 mg novobiocin sodium salt into 1 L deionized water. Filter sterilize the solution through 0.45 µm membrane. Add 2.5 mL novobiocin solution to 225 mL prepared *Shigella* broth.
Novobiocin sodium salt (Sigma-Aldrich #N1628 or equivalent)	50 mg	
Deionized water	1 L	

Trypticase Soy Broth (TSB)

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Trypticase peptone	17 g	Dissolve ingredients with heat in deionized water. Dispense 225 mL into 500 ml Erlenmeyer flasks. Autoclave at 121 °C for 15 minutes. Final pH, 7.3 ± 0.2. For trypticase soy broth without glucose, prepare as above, but omit 2.5 g glucose.
Phytone peptone	3 g	
NaCl	5 g	
K_2HPO_4	2.5 g	
Glucose	2.5 g	
Deionized water	1 L	

Trypticase Soy Broth (TSB) with Yeast

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Trypticase soy broth	30 g	Dissolve ingredients in deionized water. Autoclave at 121 °C for 15 minutes. Final pH, 7.3 ± 0.2
Yeast extract	6 g	
Deionized water	1 L	

Universal Pre-Enrichment Broth

Source: U.S. FDA Bacteriological Analytical Manual

Ingredients	Amount	Instructions
Tryptone	5 g	Dissolve ingredients with heat in deionized water. Autoclave at 121 °C for 15 minutes. Final pH, 6.3 ± 0.2.
Proteose peptone	5 g	
KH_2PO_4	15 g	
Na_2HPO_4	7 g	
NaCl	5 g	
Dextrose	0.5 g	
$MgSO_4$	0.25 g	
Ferric ammonium citrate	0.1 g	
Sodium pyruvate	0.2 g	
Deionized water	1 L	

UVM Broth, Modified

Source: USDA-FSIS Microbiology Laboratory Guidebook

Ingredients	Amount	Instructions
Proteose peptone	5 g	Dissolve ingredients in deionized water. Autoclave at 121 °C for 15 minutes. DO NOT OVERHEAT; COOL AT ONCE AFTER REMOVAL FROM THE STERILIZER. IF THE MEDIUM BLACKENS OR DARKENS, IT HAS BEEN OVERHEATED AND MUST BE DISCARDED. Store in the refrigerator. Final pH, 7.2 ± 0.2 at 25 °C.
Tryptone	5 g	
Lab Lemco powder (Oxoid)	5 g	
Yeast extract	5 g	
NaCl	20 g	
KH_2PO_4	1.35 g	
Na_2HPO_4	12 g	
Esculin	1 g	
Nalidixic acid (2% in 0.1 M NaOH)	1.0 mL	
Acriflavine	12 mg	
Deionized water	1 L	

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

Appendix B. PCR Contamination Control

Appendix B. PCR Contamination Control

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.

BAX Systems with automated detection avoid 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. See Decontamination procedures below.

Preventing PCR Contamination

In order to prevent contamination, always follow these best practices when working in the laboratory.

Work Area Preparation

- Before entering the work area, remove and dispose of any gloves you may already be wearing. Put on new gloves when entering and dispose of them before leaving the work area.
- Clean the work area and all materials and supplies before and/or after use with 10% hypochlorite bleach solution, rinse with deionized water or 70% ethanol solution and blot dry.
- A biohood in the work area is ideal. Make sure to use the UV light for 30 minutes before and/or after your use of the biohood. Lab tools and other equipment in the biohood should be rotated, if necessary, to ensure complete exposure to the UV light.

Sample Preparation

- Use barrier pipette tips for all pipetting events, if possible.
- Use new cluster tubes and caps for each sample.
- Change or clean gloves immediately before working with reagents.
- Review best practices procedures for loading samples, stressing the importance of ensuring the instrument has reached the load temperature before beginning to hydrate PCR tablets.

Materials Storage and Cleaning

- Store optical caps and cluster tube caps in a sealable container, such as a zip-lock bag or drawer. Never store optical caps and cluster tube caps in close proximity to the PCR tube rack or other items that are routinely placed in the PCR instrument.
- Clean the PCR tube rack and other items that are routinely placed in the PCR instrument with 10% hypochlorite bleach solution, rinse with deionized water or 70% ethanol solution and allow to dry after every use.
- Spray cooling blocks with 10% bleach, rinse with deionized water or 70% ethanol and blot dry before returning them to the refrigerator.
- Before performing calibration or if PCR product contamination is suspected, autoclave the pipettes according to the manufacturer's directions.

Materials Disposal

- DO NOT OPEN TUBES AFTER AMPLIFICATION. This will ensure that no amplified material is introduced into your work area.
- Dispose of used PCR tubes in a dedicated container that can be immediately autoclaved or in a designated container with bleach. Always treat tubes removed from the BAX System instrument as positive enrichments or other potential sources of contamination.
- Discard barrier pipette tips in a plastic bag, autoclave the tips, and dispose of the bag after each use of the preparation area.

Identifying PCR Contamination

If you suspect that your lab is contaminated, prepare a set of negative controls (blank lysates) and run a full process in the BAX System instrument. This “blank” acts as a negative control on the process. A positive result for the “blank” indicates contamination.

Collect environmental swab samples from a variety of areas around the lab (metal racks, decapping tools, cooling blocks, drawer handles, pipettor tips, etc.). Record the places from which environmental samples are collected; if contamination is observed at a location, these same areas should be sampled again once decontamination has been completed.

When collecting and processing samples, always use a new, unopened BAX System kit (including protease, lysis buffer, PCR tubes and caps) and new, unopened boxes of supplies (including gloves, pipette tips and cluster tubes). The best aseptic technique should be used when processing environmental swab samples to ensure the highest integrity data.

Using the following protocol to collect and process the environmental swab samples:

1. Hydrate sterile swab in 500 µL diluent* (peptone water, DI water, lysis buffer, etc.).
2. Swab the sample area.
3. Place swab back into individual diluent* tubes and mix/vortex to agitate samples.
4. Transfer 5 µL of this solution into prepared BAX System lysis reagent (lysis buffer + protease).

   Note: *If using the BAX System real-time PCR assays for E. coli or STEC, transfer 20 µL solution into lysis reagent.*

5. Follow the BAX System lysis procedure for the target organism to heat and cool samples and run a full process in the BAX System instrument.

   ***** *If using the BAX System real-time PCR assays for STEC, use sterile mTSB or BAX System MP media as the diluent.*

Removing PCR Contamination

If contamination should occur, the work area must be thoroughly cleaned. During the cleaning process, it is important to change gloves, wipes and other decontamination materials often to prevent spreading PCR product to new areas via these materials.

While wearing gloves and a lab coat, perform the following procedures under sterile conditions:

- Wipe down the lab environment with 20% bleach solution*, followed by 70% ethanol solution. If possible, let the bleach solution sit for 10 minutes before wiping up with a dry wipe. If possible, irradiate the surfaces with a UV light source, being sure that all sides of the surface are exposed to UV light for at least 30 minutes.
  - Wipe the outside of all the equipment and the keyboard and mouse for each work station.
  - Wipe all cabinets, countertops, refrigerator handles, door handles, and anything else that is touched by hands in the lab environment.
  - Wipe heating blocks and timers.
  - Wipe all materials on sample prep areas, including pipettors, stands, table tops, etc.
- For electrical equipment, wipe down the surface with a cloth dampened with the bleach solution followed with a 70% ethanol rinse to prevent damage to the equipment.
- Soak smaller items in 20% bleach solution for at least 5 minutes, then rinse with water and allow to air dry.
  - Soak cluster tube racks and PCR racks.
  - Soak scissors, tweezers, cooling and heating block inserts, and two styles of decappers.
- Discard all disposable items (pipette tips, buffers and other solutions, etc.) that have been opened or partially used.
- Autoclave pipettes and all other ancillary equipment that can tolerate autoclaving, according to the manufacturer's directions.
- Mop or wipe all floors with bleach solution before leaving the lab after completing all other decontamination steps.

** A 10% bleach solution may also be used for decontamination in accordance with your laboratory SOP but may require repetition of the cleaning to assure removal of all amplicon.*

Monitoring PCR Contamination

To help prevent subsequent PCR contamination incidents, a laboratory may opt to incorporate a routine amplicon monitoring program that follows a procedure similar to established environmental monitoring programs. In the case of an amplicon monitoring program, the environmental target is DNA rather than viable bacteria.

- Routine amplicon monitoring programs may include a weekly rotation of sampling from representative areas throughout the lab, both before and after standard cleaning practices, as a means of monitoring the effectiveness of cleaning.
- If contamination is confirmed in any of these areas, a more thorough and rigorous monitoring procedure should be implemented until it is determined that contamination no longer exists.

Appendix C. Confirmation Utility

Appendix C. Confirmation Utility

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

Good laboratory practice suggests that you not use the same method to both screen a sample and confirm its results. When using the BAX System to screen a sample, any desired confirmation is usually performed though biochemical methods. Conversely, when screening with traditional biochemical methods, you can use the BAX System to identify suspect target colonies on selective agar plates. The sample preparation steps vary slightly from those used with the screening protocol; otherwise, the method is the same. The only additional supplies you need are plastic disposable inoculating needles and sterile diluent, such as 0.1% peptone water.

To identify suspect colonies, follow these steps:

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

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

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

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

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

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

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

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

Atypical Colony Confirmation Results

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

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

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

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

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

Appendix D. Troubleshooting

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

For additional technical assistance, contact your local distributor or call Hygiena Technical Support at 800-863-6842 / 888-494-4362 or contact technical support from the Hygiena website under support (www.hygiena.com/support).

Troubleshooting the Instrument

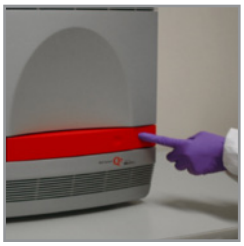
When the instrument is ready for use, the “Power” light on the front panel is displayed. If a red “Error” light displays instead, try these steps:

1. Press on the instrument door to ensure that it is closed.	
2. If the error light continues to display, open the instrument door and pull on the heated cover to verify that is fully forward. Close the instrument door.	

3. If the error light continues to display, check the monitor. If you don't see the desktop for the Windows operating system, turn off power to the instrument and wait for the monitor to display the desktop. Then power on the instrument.

4. If the error light continues to display, check the USB cable connections. Verify that one end is connected to the back of the instrument and the other to the computer.

5. If the error light continues to display, power off the instrument, wait 30 seconds, then power it back on.



6. If the error light continues to display, contact Hygiena Technical Support or your distributor for technical assistance.

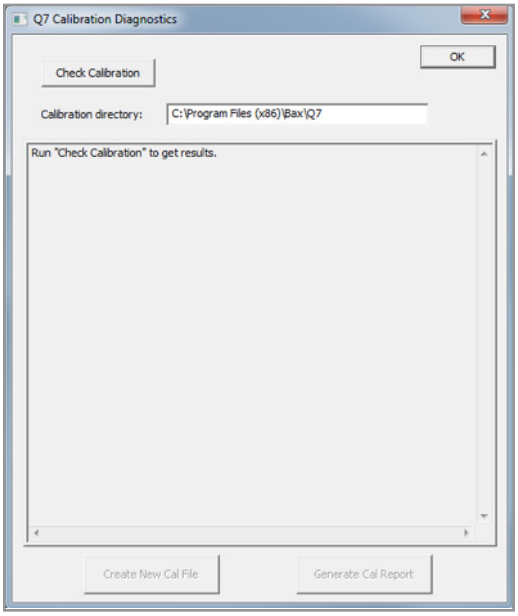
Troubleshooting Calibration

Due to the particular nature of the calibration process, any minor deviation from the protocol described under “Calibrating the Instrument” in Chapter V: BAX System Hardware may result in a calibration failure. If the calibration process fails, you can make one or more of the following adjustments and re-try performing the calibration.

- Check the failed calibration plate for droplets on the top film. If you see any, flick the plate so that the droplets return to the wells.
- Allow the calibration plates to reach room temperature.
- Remove any smudges or dust particles from the halogen bulb. Wear gloves when handling the bulb to avoid leaving fingerprints.
- Remove the halogen bulb from the socket, rotate it 180° and re-insert it into the socket.
- Replace the calibration plates if one or more wells have significantly less liquid than the other wells.

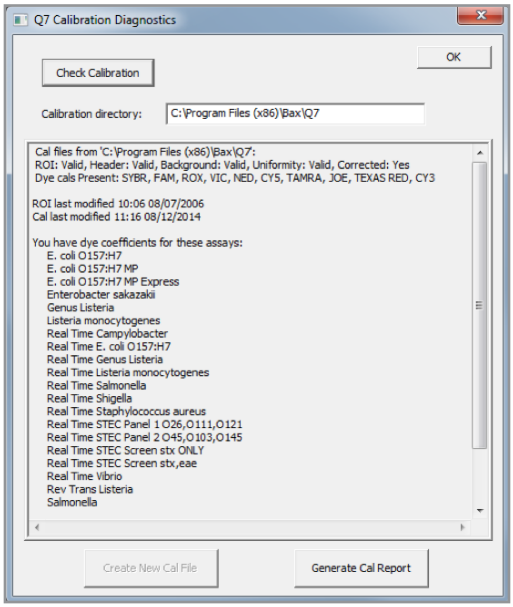
Calibration Diagnostics

After installing or re-installing BAX System software, or if you suspect that there is a problem with the instrument, you can run calibration diagnostics to determine the status of your calibration files. This diagnostics feature also allows you to create some types of calibration files, if necessary.



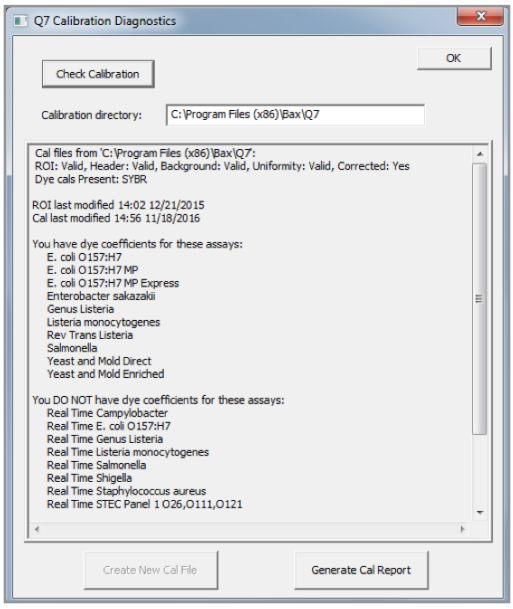
To run calibration diagnostics:

1. From the menu bar, select DIAGNOSTICS > CALIBRATION to open the Q7 Calibration Diagnostics window.
2. Click the **CHECK CALIBRATION** button to create a list of the valid calibration files.
3. After the calibration diagnostic has been run, the results will appear in the calibration window.



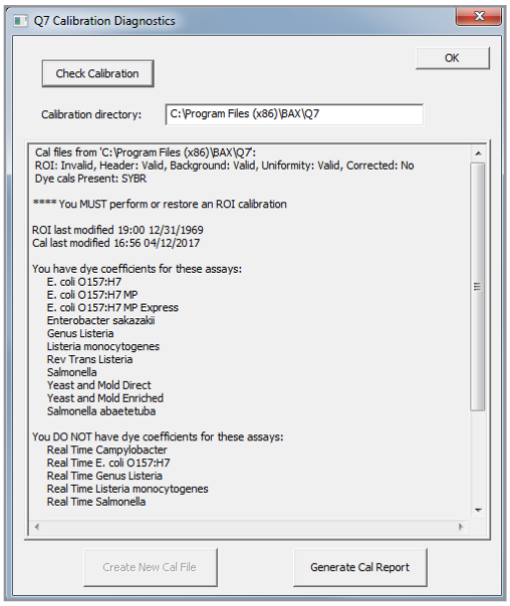
If the results state that you have coefficients for both standard and real-time assays (and does not list assays for which you do not have coefficients), then all calibration files are complete and properly installed to run standard, 24E and real-time assays. No further calibration is needed.

Calibration Diagnostics (Continued)



If the results state that you have coefficients for standard (melt) assays but do not have coefficients for real-time assays, then the instrument is not calibrated to run any real-time assay. The instrument is capable of running standard and 24E assays only.

To add coefficients to run real-time assays, follow the protocol described under “Calibrating the Instrument” in Chapter V: BAX System Hardware to complete calibration.



If the results state that you do not have an ROI calibration, then the instrument is not calibrated to run any standard or real-time assays.

To calibrate the instrument for ROI or real-time assays, follow the protocol described under “Calibrating the Instrument” in Chapter V: BAX System Hardware to complete calibration.

Troubleshooting Indeterminate or Error Results

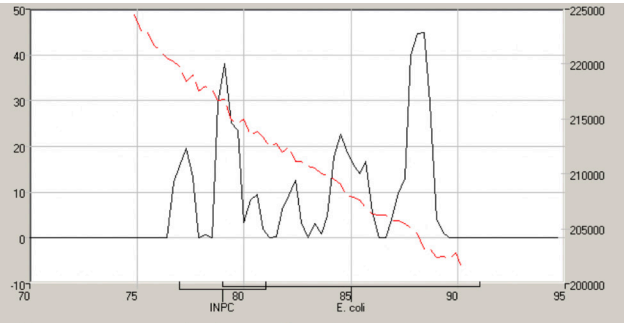
	Yellow	=	Indeterminate result
	Yellow (?) with red slash	=	Signal error

Indeterminate and error results are most commonly caused by

- Missing PCR tubes
- Non-specific reactions
- Straight line signal
- Exponential decay

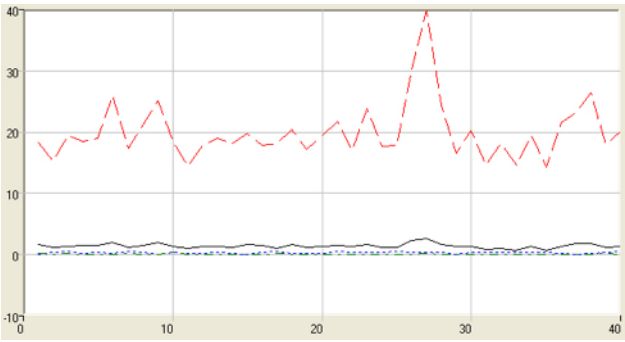
In these situations, you can examine the shape of the melt curve and its Y-axis scale (standard assays) or the attributes of the amp plot (real-time assays) to help diagnose the problem.

Missing PCR Tubes



Melt Curve Diagnosis (standard assays):

The Raw view shows a jagged pattern of multiple steps. The Processed view shows system noise at low signal levels.

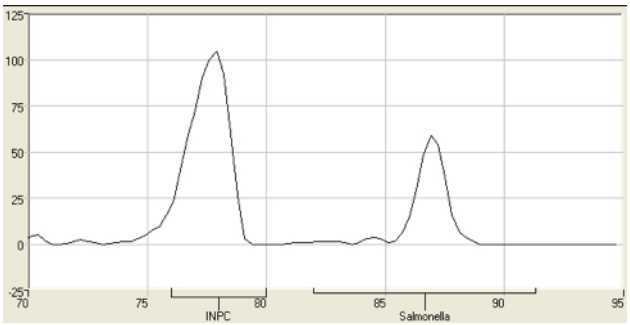


Amp Plot Diagnosis (real-time assays):

All target curves and the IPC curve appear as jagged lines (system noise). The IPC may also appear as a straight horizontal line, and no target curves appear.

Cause	Solution
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.	Although not evident in the mislabeled wells, the data has been captured and does exist in the file. 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

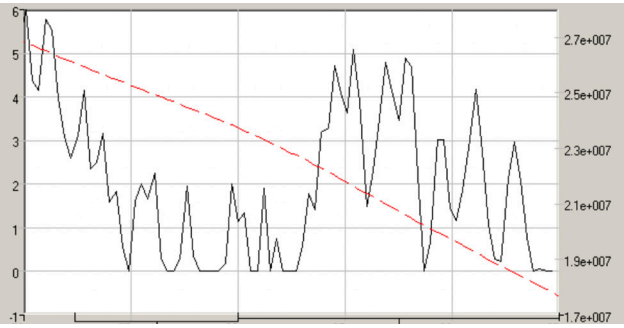


Melt Curve Diagnosis –

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

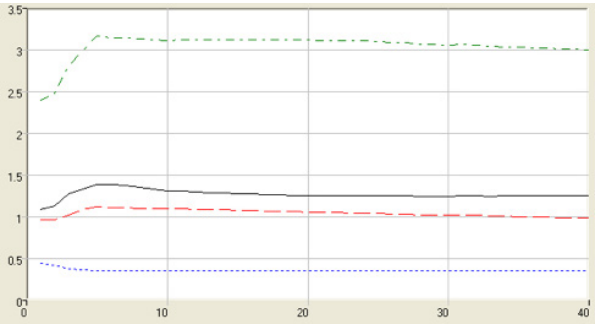
Cause	Solution
Samples were not loaded into the BAX System cycler immediately after hydrating PCR tablets.	Repeat the assay from last enrichment. Do not begin to hydrate PCR tablets with lysate until the cycler has reached the correct load temperature and the “Ready for Rack Load” prompt appears. Load samples into the instrument immediately after hydrating tablets.
Samples were not adequately chilled to 2 to 8 °C after heating for lysis.	Repeat the assay from last enrichment. Ensure that all cooling blocks are chilled to 2 to 8 °C before use. Keep samples in the cooling block for a full 5 minutes before using to hydrate PCR tablets.
After lysis, samples were not maintained at 2 to 8 °C before loading into the cycler.	Repeat the assay from last enrichment. Ensure all cooling block inserts have been chilled to 2 to 8 °C before use, and do not remove cooling blocks from the refrigerator until they are needed to keep them as chilled as possible. Finish using the cooling blocks within 30 minutes of removing inserts from the refrigerator. ***Note:*** *If you are using the E. coli O157:H7 MP assay, ensure that the cooling block for PCR tubes is maintained at -20 °C.*

Straight Line Signal



Melt Curve Diagnosis –

The Raw view shows a negatively sloped line. The Processed view is blank or may show system noise.



Amp Plot Diagnosis (real-time assays):

The IPC and target curves appear flat. The IPC curve reports a Ct value of 0.0.

Cause	Solution
Second lysis step (95 °C) was not performed properly.	Check that the heater is reaching 95 °C by inserting a thermometer into one of the two threaded holes in the block. Repeat the assay from last enrichment, confirming that the lysis tubes heat for a full 10 minutes at 95 °C.
Missing tablet – can happen when the tablet sticks to the pipette tip.	Repeat the assay from last enrichment. Check that the PCR tablet does not stick to the pipette tip and get discarded when the tip is ejected.
Dry tablet – can happen when pipettor is clogged or tips are loose.	Repeat the assay from last enrichment. Check that the pipettor is dispensing accurately, and that the tips are secure.
Powder from gloves is interfering with PCR	Use only powder-free gloves for handling samples. Repeat assay from last enrichment.

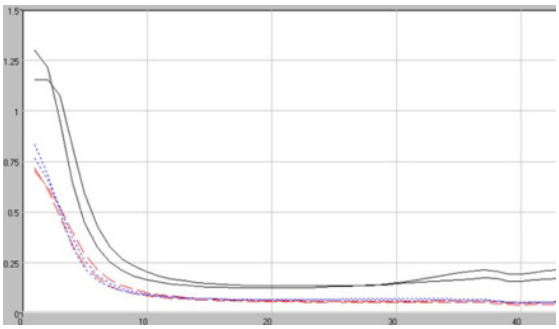
Straight Line Signal (Continued)

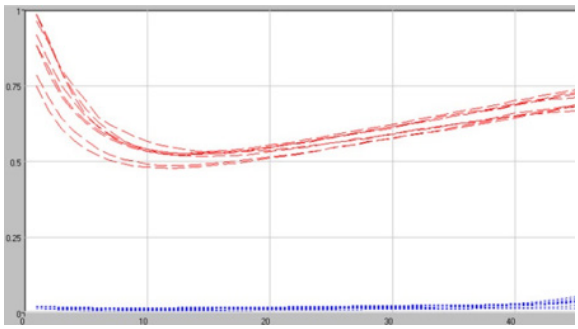
Cause	Solution
Samples were run in “Detection only” mode instead of “Run Full Process.” You can verify this by selecting VIEW > EDIT RACK INFO and checking the field for PCR Time in the lower pane. If the field is blank, no amplification occurred.	Repeat the assay from the last enrichment. Take care to select Run Full Process.
PCR Inhibition or interference such as from a high concentration of detergent/disinfectant which can interfere with PCR (can happen when sampling from recently sanitized areas without using a neutralizing buffer as wetting agent).	Repeat the assay from the last enrichment to rule out sample prep errors. If straight line signal persists, contact Hygiena Technical Support for assistance.
The sample type or enrichment protocol being tested has not been validated.	Repeat the enrichment according to the protocol described in the BAX System User Guide. If you are testing a non-validated sample type, call Hygiena Diagnostics Support for assistance.

Exponential Decay

Amp Plot Diagnosis (real-time assays):

The IPC and target curves begin relatively high, then drop and remain flat or rise slightly for the rest of the reaction, which can cause an indeterminate result.

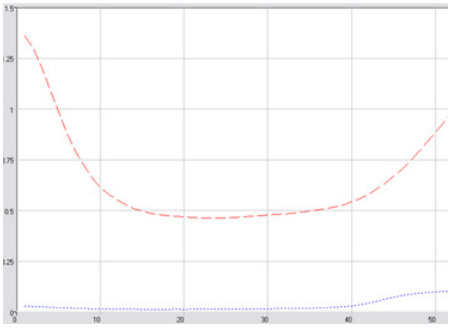




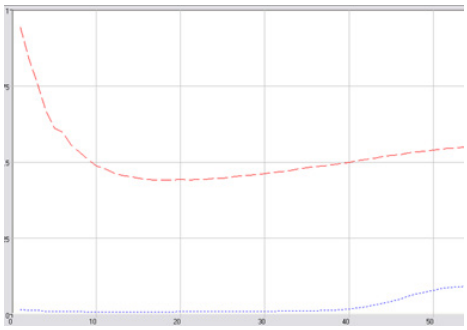
Exponential Decay (Continued)

Cause	Solution
PCR tablets were exposed to air for more than 15 minutes before being hydrated with lysate.	Repeat the assay from the last enrichment using new PCR tubes. Do not uncap PCR tubes until you are ready to hydrate the tablets. Only uncap one strip of PCR tubes at a time. OR Repeat the assay from the last enrichment using new PCR tubes. After hydrating PCR tablets with lysate, vortex (15-30 seconds) and centrifuge the plate to ensure all the liquid is at the bottom of the tube.
PCR tablets were not fully hydrated (insufficient volume of lysate transferred to PCR tubes).	Check that all pipettors are delivering the correct volume of lysate to the PCR tubes, then repeat the assay from the last enrichment using PCR tubes from a new BAX System kit.
PCR tubes were exposed to humidity during storage or were stored improperly.	Discard any remaining PCR tubes from the kit, and repeat the assay from the last enrichment using PCR tubes from a new BAX System kit. Before storing PCR tubes, re-seal the PCR tablet bag tightly. If storing PCR tubes in an open kit for more than 3 weeks, seal the bag of PCR tubes into a larger bag with desiccant or store at 4 °C in a desiccation unit, if possible.

Note: *Some signs of exponential decay can also appear in samples that return positive or negative results (not indeterminate). These signs do not affect the validity of the BAX System results, and action can be taken based on the plus/minus determinations. Note: When performing quantification, any samples that show exponential decay should be rerun from the lysate (as the decay impacts Ct values used for quantification).*



Positive result with exponential decay



Negative result with exponential decay

Troubleshooting Process Interruptions

In some cases, a full process run in the BAX System 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.

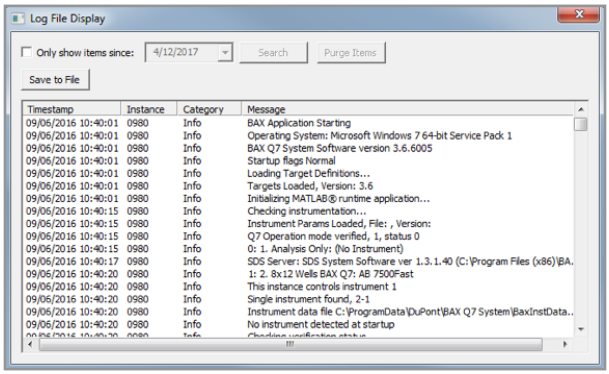
Note: *The Detection Only option SHOULD NOT be used to re-analyze samples for which results have already been displayed. To reanalyze results that have already been produced, select OPERATION > REANALYZE WELLS and follow the screen prompts.*

Note: *This option is not available for real-time assays.*

Review the Log File

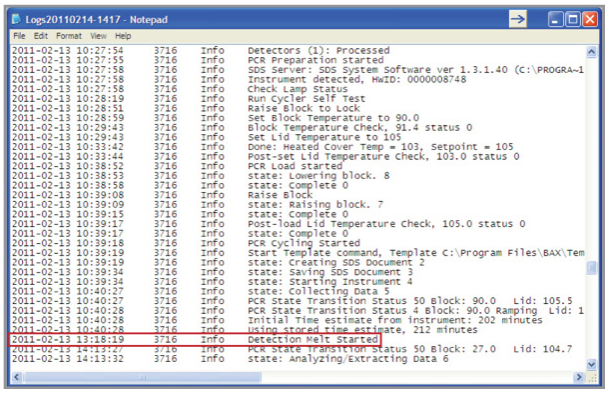
From the menu bar, select DIAGNOSTICS > VIEW LOG FILE to open log file display.

Locate the log file entry with the date and time of the interrupted process run.



Double-click on the log file to view details.

Note: *You can also click the SAVE TO FILE button to save a text version of the log file to your computer desktop.*



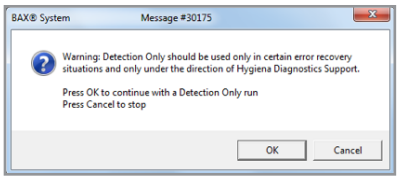
Locate the line in the log file that states “Detection Melt Started” or "Melt in Progress". If the “Detection Melt Started” line appears in the text file, follow the instructions below to re-run samples using the Detection Only operation.

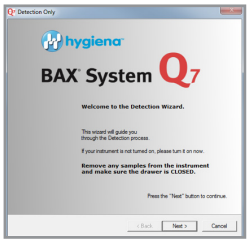
If this line does not appear in the text file, new PCR tables must be hydrated from the saved lysate to re-run the full process in the BAX System instrument.

Perform Detection Only

Note: *This operation should be run* ***as soon as possible*** *after the process interruption occurs. Contact Hygiena Diagnostics Support for guidance before performing Detection Only.*

1. Power on the BAX System Q7 instrument. From the menu bar, select DIAGNOSTICS > DETECTION ONLY to launch the Detection Wizard. You will get a warning dialog to confirm that you want to do a Detection Only run. Select **OK** to get to the run wizard.
2. Remove any samples from the instrument and close the sample drawer. Click **NEXT** .

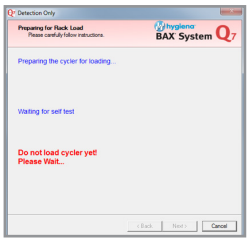




3. A new screen automatically appears as the instrument heats to its set temperatures. The red color in the banner indicates that it is a Detection Only run.

Note: *DO NOT LOAD SAMPLES into the instrument until prompted to do so.*

4. Follow the screen prompts to perform detection. The process completes in approximately 1 hour.



BAX SYSTEM LIMITATION OF WARRANTY AND LIABILITY

BAX SYSTEM LIMITATION OF WARRANTY AND LIABILITY

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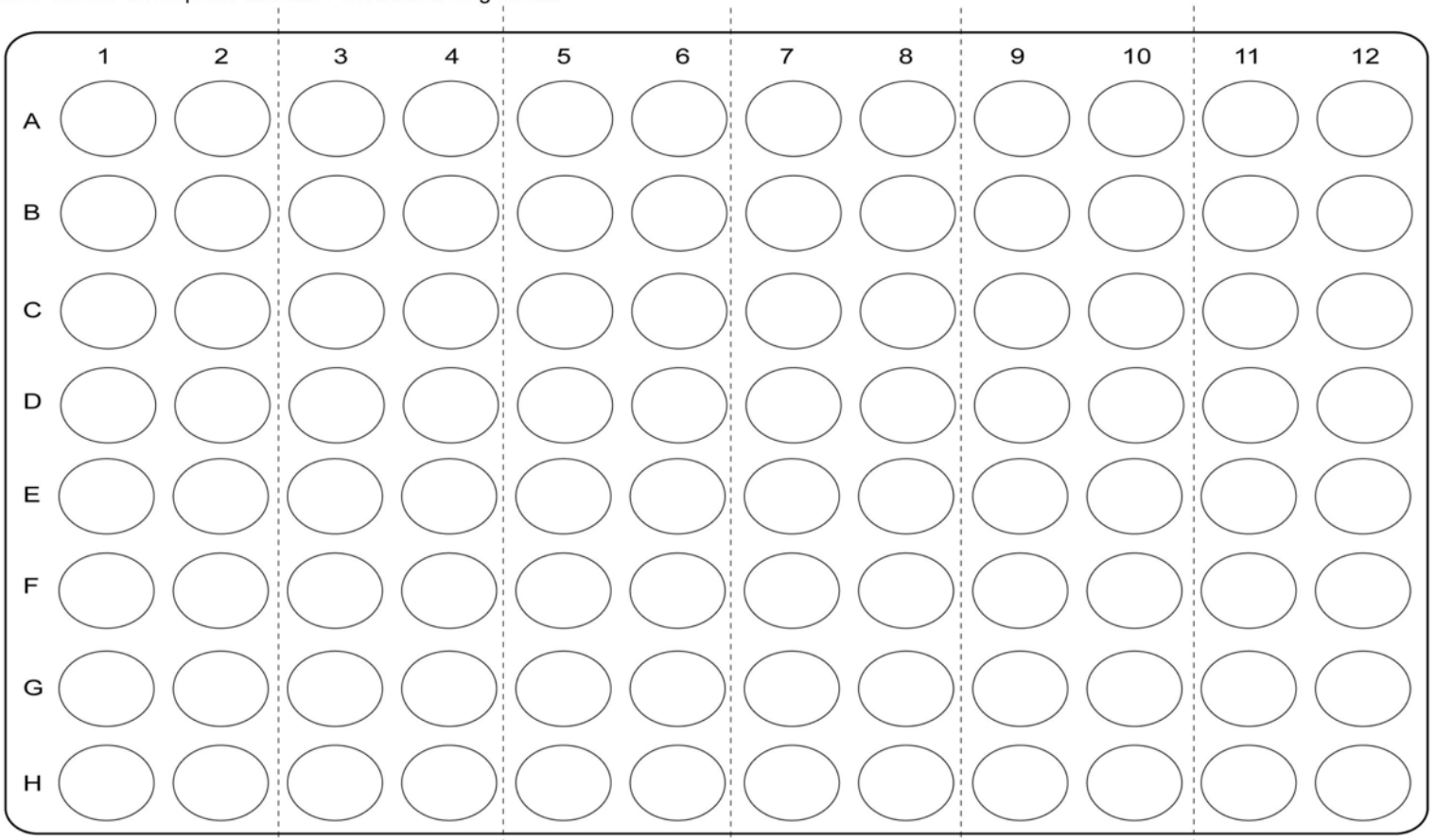
BAX® System Sample Tracking Sheet

Organism: _____

Date: _____

Enter one sample ID number into each circle.
These sheets correspond with the PCR rack arrangement.



Date lysis: _____ Kit lot # _____ Media lot # _____

Date PCR: _____ Exp. date: _____ Exp. date: _____

Technologist: _____

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