



BAX® System Real-Time PCR Assay

E. coli O157:H7 Exact +S

Product Number KIT2043



KIT CONTENTS

- 96 PCR tubes with lyophilized pellet (2 bags of 6 x 8 strips)
- 96 flat optical caps (12 x 8 strips)
- 1 bottle of protease (400 µL)
- 2 bottles of lysis buffer (12 mL each)

INTENDED USE

Overview

Food processors and associated laboratories can use the BAX® System as a quick and reliable method for detecting *E. coli* O157:H7 in relevant food samples. This real-time PCR assay was designed to report yes/no results for the presence of *E. coli* O157:H7 at concentrations as low as 10⁴ cfu/mL after enrichment. With a processing time of approximately 55 minutes on the BAX® System Q7 instrument, the method returns results with an accuracy that is comparable to culture methods, but with a significantly faster time to result.

BAX® Systems are designed for use by qualified lab personnel who follow standard microbiology laboratory practice, including the safe handling and disposal of potentially pathogenic materials. The laboratory must comply with good laboratory practice (see ISO 7218 standard).

This kit offers the same reliable results as the BAX® System Real-time *E. coli* O157:H7 Exact assay (KIT 2039, PTM 102003), but with an added "S" target. The "S" target is an indicator marker found in samples recovered from environments conducive to *E. coli* O157:H7 growth.

AOAC PTM 032602 certification includes the detection and colony confirmation of the *E. coli* O157:H7 target only.

Field of use

Data obtained from the BAX System should not be used for human diagnostic or human treatment purposes. Equipment is not approved by the United States Food and Drug Administration or any other US or non-US regulatory agency for use in human diagnostics or treatment. The BAX System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program.

Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under the supervision of technically qualified persons.

PRINCIPLE OF THE METHOD

See the BAX System User Guide for an overview of how the BAX System method uses automated, real-time Polymerase Chain Reaction (PCR) technology.



MATERIALS

- BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact +S, Product No. KIT2043
- BAX System start-up package (equipment and supplies for up to 192 tests)
 - BAX System Q7 cycler/detector and computer workstation
 - Heating blocks with inserts* capable of maintaining $37 \pm 2^\circ\text{C}$ and $95 \pm 3^\circ\text{C}$
 - Cooling blocks with inserts*
 - PCR tube holder
 - Vortex Mixer
 - Capping/decapping tools
 - Adjustable mechanical pipettes (5-50 μL ; 20-200 μL)
 - Repeating pipette
 - Multi-channel pipettor (8 channels – 5-50 μL)
 - Cluster tubes with caps and racks
 - Pipette tips with aerosol barriers
 - Powder-free nitrile gloves
 - Homogenizer/Stomacher (Seward Stomacher® 3500W or equivalent) with bags
 - Incubator capable of maintaining directed enrichment temperatures within $\pm 2^\circ\text{C}$
- Meat Sampling cloth such as Fremonta MicroTally™ Manual Sampling Device (MSD) cloth (MT-S100) or similar

*The Automated Thermal Block (Product No. MCH2023) may be used instead of heating and cooling blocks.

STORAGE AND SHELF LIFE

- Reagents and PCR tubes with tablets should be kept refrigerated at 2 to 8°C . Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- After protease has been added to the lysis buffer, the shelf life of the solution is 2 weeks when stored at 2 to 8°C .
- If storing PCR tubes with tablets in an open kit for more than 3 weeks, seal the Mylar® bag of PCR tubes into a larger bag with desiccant or store at 4°C in a desiccation unit, if possible.

SAFETY PRECAUTIONS

The BAX System method includes sample enrichment procedures that nourish the growth of potential pathogens to detectable levels. Because pathogens can cause human illness, appropriate safety precautions must be taken and personal protective equipment must be worn when handling samples, media, reagents, glassware and other supplies and equipment that could be contaminated with potentially pathogenic bacteria.

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

Sharps Disposal: All sharps, including disposable plastic needles, must be disposed of immediately after use in rigid, puncture-resistant sharps containers that comply with local regulations. Sharps containers should be positioned within arm's reach of the work area, filled to no more than three-quarters capacity, and sealed before disposal according to institutional biohazardous waste protocols. Do not dispose of sharps in regular waste receptacles.

Reagents used with the BAX System assays should pose no hazards when used as directed. Before using this assay, please review the Safety Data Sheet (SDS) included with your BAX System purchase; it is also available at



www.hygiena.com/documents. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

Before using this assay for the first time, install the most current version of the BAX System software (links to software downloads can be found on hygiena.com), then run a calibration report to check that 'Real Time *E. coli* O157:H7 Exact +S' appears in the list of calibration files. See "Troubleshooting Calibration" in the BAX System User Guide for details.

If the report list does not contain 'Real Time *E. coli* O157:H7 Exact +S', you must recalibrate the Q7 instrument to load the required dyes. Be sure to allow enough time to complete the calibration (about 1.5 to 2 hours) before starting the assay. For instructions and tips on calibrating the instrument, see the BAX System User Guide.

ENRICHMENT PROTOCOL

1. Prepare Enrichment Broth

MP media: Dissolve 22.5 g BAX System MP media in 1 L distilled water and mix. Do not boil. Adjust the pH to a final value of 7.2 ± 0.2 at 25 °C, then autoclave at 121 °C for 15 minutes. Broth can be stored for up to 2 weeks at 2 to 8°C.

For all other non-proprietary enrichment media: prepare enrichment broth according to the manufacturer's instructions. See the BAX System User Guide for common enrichment media recipes.

2. Collect and enrich samples:

- *Raw ground beef:*

For **375 g** – Homogenize 375 g sample with 1.5 L pre-warmed (45 °C) BAX System MP media and stomach at 230 rpm for 1 minute. Incubate at 42 °C for 8-24 hours.

For **325 g** – Homogenize 325 g sample with 975 mL mTSB + caa (modified TSB with 10 g/L casamino acids) and stomach at 230 rpm for 1 minute. Incubate at 42 °C for 15-24 hours.

- *Beef trim:*

For **375 g** – Mix 375 g sample with 1.5 L pre-warmed (45 °C) BAX System MP media and stomach at 230 rpm for 1 minute. Incubate at 42 °C for 8-24 hours.

For **325 g** – Homogenize 325 g sample with 975 mL mTSB + caa (modified TSB with 10 g/L casamino acids) and stomach at 230 rpm for 1 minute. Incubate at 42 °C for 15-24 hours.

- *Leafy Greens:*

For **375 g** – Combine 375 g sample with 1.5 L pre-warmed (42 °C) BAX System MP media and hand massage for 1 minute. Incubate at 42 °C for 8-24 hours.

- *Sampling Cloths such as MicroTally® (375 g Beef Trim portion):*

MP Media: Combine one sampling cloth with 200 mL pre-warmed (42 °C) MP media. Stomach for 1 minute at 230 rpm and incubate at 42 °C for 8-24 hours.

mTSB + caa (modified TSB with 10 g/L casamino acids) media: Combine one sampling cloth* with 200 mL mTSB + caa media. Stomach for 1 minute at 230 rpm and incubate at 42 °C for 8-24 hours.

**NOTE: For meat sampling cloths enriched in mTSB + caa, 25 mL of chilled nBPW is added to each cloth at the time of sampling, per USDA FSIS Sampling Guidance (https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/10010.1.pdf). Meat sampling cloths enriched in MP media do not require the addition of nBPW at the time of sampling.*



TEST PROTOCOL

1. Prepare Equipment

- 1.1 Turn on the heating blocks to 37 °C and 95 °C*.
- 1.2 Make sure cooling blocks are chilled to 2 to 8 °C*.

*If using the Automated Thermal Block, follow the instructions in the Automated Thermal Block User Guide for running the 'gram negative' program.

- 1.3 Power on the Q7 instrument and launch the BAX System application.
- 1.4 Create a rack file (see User Guide for details).

4. Perform Lysis

- 4.1. Break cluster tubes apart.
- 4.2. Label and arrange cluster tubes in the rack according to the rack file.
- 4.3. Prepare lysis reagent by adding 150 µL protease to one 12 mL bottle of lysis buffer.
- 4.4. Transfer 200 µL lysis reagent to each cluster tube.
- 4.5. Transfer 20 µL enriched sample to the corresponding cluster tube.

Note: *Enrichments can be stored at room temperature (18 to 25°C) until test results have been reviewed and accepted (up to 4 hours, unless otherwise validated internally).*

- 4.6. Heat at 37 °C for 20 minutes.
- 4.7. Heat at 95 °C for 10 minutes.
- 4.8. Cool at 2 to 8 °C for at least 5 minutes.

5. Hydrate PCR Tablets

- 5.1. Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.
- 5.2. Place a PCR tube rack onto a chilled (2 to 8 °C) PCR cooling block.
- 5.3. Arrange strips of PCR tubes according to your rack file.
- 5.4. Remove the caps from the first strip of tubes with the decapping tool.
- 5.5. Transfer 30 µL lysate (from Step 4.8) into PCR tubes, then seal with flat optical caps.
- 5.6. Repeat with the remaining strips of PCR tubes until all PCR tablets have been hydrated.

Note: *PCR tablets must be hydrated and resealed immediately after removing the caps from the PCR tubes.*

- 5.7. After completing the hydration of all PCR tablets, let the PCR tubes sit in the cooling block for 10-30 minutes before loading them into the BAX® System instrument.

Note: *Do not let PCR tubes sit for more than 30 minutes.*

6. Amplify and Detect

- 6.1. At the 'Ready for Rack Load' prompt, click the NEXT button and open the instrument drawer.
- 6.2. Place the rack of PCR tubes over the wells in the drawer and check that the tubes are seated correctly.
- 6.3. Close the drawer and click the NEXT button to begin automated processing.



7. Review Results

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

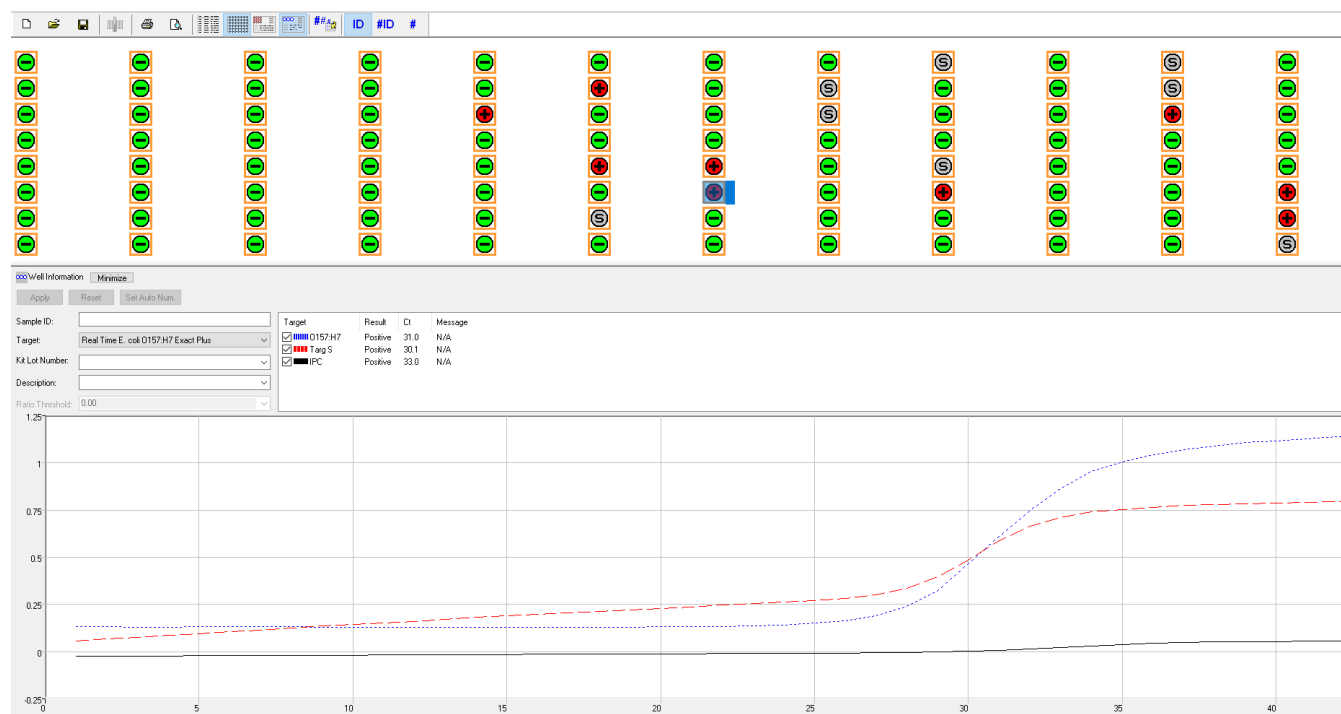


Figure 1: Screenshot from BAX Q7 Software showing Real Time E. coli O157:H7 Exact +S menu option. The top panel is the rack-view grid of color-coded icons with O157:H7 positive wells shown as red (+), S-target-only positive wells shown as Grey (s), and negative wells shown as Green (-) icons. The bottom panel shows the amplification PCR curves for the selected sample.

Three menus can be chosen for different analysis needs.

Real Time <i>E. coli</i> O157:H7 Exact +S	Real Time <i>E. coli</i> O157:H7 Exact +S -O157:H7 ONLY	Real Time <i>E. coli</i> O157:H7 Exact +S -S Marker Only
Green (-) = Negative for target organism	Green (-) = Negative for target organism	Green (-) = Negative for target organism
Grey (S) = Positive for S Marker*	Red (+) = Positive for O157:H7	Grey (S) = Positive for S Marker*
Red (+) = Positive for O157:H7	Yellow (?) = Indeterminate result	Yellow (?) = Indeterminate result
Yellow (?) = Indeterminate result	Yellow (?) with red slash = Signal error†	Yellow (?) with red slash = Signal error†
Yellow (?) with red slash = Signal error†		

***Positive for S Marker:** Increasing frequency and intensity of positive results for the S target in the absence of the *E. coli* O157:H7 target can serve as an early warning sign. Such occurrences suggest a heightened risk of future *E. coli* O157:H7 contamination in subsequent tests of similar samples from the same environment.

†Refer to the troubleshooting section in the User Guide for assistance.



CONFIRMATION

If desired, BAX method enriched samples can be confirmed with the reference culture method appropriate for the sample type, such as:

- USDA FSIS Microbiology Laboratory Guidebook (MLG)

All samples identified as positive by the BAX System method must be confirmed in one of the following ways:

- Direct plating- Streak 50 µL of enrichment onto TC-SMAC and Rainbow agar or mRBA (Modified Rainbow agar). Incubate plates for 18-24 hours at 37 °C. Test 1-5 typical colonies from at least one selective agar plate by:
 - Latex agglutination and acceptable molecular method.
- Immuno-concentration (IMS) - Perform IMS and streak 50 µL of enrichment onto CT-SMAC and Rainbow agar or mRBA. Incubate plates for 18-24 hours at 37 °C. Test 1-5 typical colonies from at least one selective agar plate by:
 - Latex agglutination and acceptable molecular method.

If confirming a colony with the BAX method*, 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 complete 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 E. coli O157:H7 Exact +S, use MP media 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 20 µL of the cell suspension into the corresponding lysis tube containing 200 µL lysis reagent, and mix.
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."

*The BAX System Real-Time *E. coli* O157:H7 Exact +S can be used to confirm presumptive positive colonies isolated from methods other than the BAX System Real-Time *E. coli* O157:H7 Exact +S.

DISPOSAL

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



TECHNICAL ASSISTANCE

For general information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

You can also call 800-863-6842 in the US, 1- 302-695-5300 outside the US, or email diagnostics.support@hygiena.com.

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2. When used with BAX® System assays, BAX® System Equipment is warranted to be free of defects in materials, workmanship and design that may appear under normal and proper use within twelve (12) months from the installation date to the first end user. BAX® System assays are warranted to conform to the assay description under the conditions of use specified in the user documentation to the expiration date stamped on the label. BAX® System media is warranted to meet standard specifications in effect on the date of shipment. Hygiena MAKES NO OTHER WARRANTY, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF

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4. The accuracy of the BAX® System can be affected by factors over which Hygiena has no control, including, without limitation, the use of the Equipment, assays and/or media in a manner that is contrary to the conditions of use, the procedures or the instructions specified by Hygiena. Because of the large number of factors over which Hygiena has no control, Hygiena makes no promise or guarantee of the accuracy of, or results obtained from the use of the BAX® System. In particular, Hygiena disclaims any warranty or liability and assumes no responsibility whatever for the failure of the BAX® System due, in whole or in part, to user's failure to: (a) properly maintain Equipment, (b) maintain specified operating or storage conditions, (c) follow the specified instructions, or (d) use the proper



microbiological techniques consistent with the standard of care accepted in the industry for the proper collection, storage, handling and preparation of the sample.

5. Externally caused failures, such as improper sample preparation, improper storage or loading of reagents, electrical outages, or out-of-specification environmental conditions are not covered under this warranty. Equipment failures caused by spills, abuse, misuse, negligence, or improper operation are not covered by this warranty. Modifications, service or repairs by parties other than Hygiena-authorized providers are not covered by this warranty and, in fact, void this warranty. Circumstances beyond the reasonable control of Hygiena, including fire, explosions, accidents, flood, labor trouble or shortage, war, act of or authorized by any government, inability to obtain suitable material, Equipment, fuel, power or transportation, or acts of God, are not covered under this warranty.
6. The BAX® System is designed to test only for the presence of the target organisms specified in the particular assay. The BAX® System has been tested against many, but not all, strains of the target within the sample types specified in the user documentation. Hygiena, therefore, cannot and does not make any representation or warranty that the BAX® System is capable of detecting every organism in the target genus, serotype, or species in any sample source. Accordingly, the BAX® System should not be used as the sole test for the release of user's products, nor should it be used as the sole basis for determining the safety of user's products.
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