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BAX® System Real-Time PCR Assays

STEC Suite

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 091801

Screening Assay – Part KIT2021 (D14642964) for *stx* and *eae*

KIT CONTENTS
96 PCR tubes with tablets (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)

Panel 1 Assay - Part KIT2008 (D14642970) for *E. coli* O26, O111, O121

KIT CONTENTS
48 PCR tubes with tablets (1 bag 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)

Panel 2 Assay - Part KIT2009 (D14642987) for *E. coli* O45, O103, O145

KIT CONTENTS
48 PCR tubes with tablets (1 bag 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)

INTENDED USE

Food processors and associated laboratories can use the BAX® System STEC suite as a quick and reliable method for detecting pathogenic Shiga toxin-producing *E. coli* (STEC) in a variety of foods. Each real-time PCR assay in the STEC suite is designed to report clear yes/no results at concentrations as low as 10^4 cfu/mL after enrichment. The screening assay detects the STEC virulence genes (stx_1, stx_2 and *eae*) with a simple, single-stage enrichment; two panel assays can then use the same lysate to determine if an STEC-screening positive is also positive for one of the top six STEC serogroups (*E. coli* O26, O45, O103, O111, O121, and O145). With a processing time of approximately 55 minutes in the BAX® System Q7 instrument, the method returns results comparable to culture methods, but with a significantly faster time to result.

BAX® Systems are designed for use by qualified lab personnel who follow standard microbiology laboratory practice, including the safe handling and disposal of potentially pathogenic materials.

Field of use: Data obtained from the BAX® System should not be used for human diagnostic or human treatment purposes. Equipment is not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. The BAX® System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under supervision of technically qualified persons.

PRINCIPLE OF THE METHOD

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

MATERIALS

BAX® System Real-Time PCR Assays – STEC suite
STEC Screening assay for *stx* and *eae* (Part KIT2021[D14642964]);
STEC Panel 1 assay for *E. coli* O26, O111, O121 (Part KIT2008 [D14642970]); or
STEC Panel 2 assay for *E. coli* O45, O103, O145 (Part KIT2009 [D14642987])

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±2ºC and 95±3ºC
- Cooling blocks with inserts*
- PCR tube holder
- Capping/decapping tools
- Adjustable mechanical pipettes (5-50 µL; 20-200 µL)
- Repeating pipette
- Multi-channel pipette (8 channels – 5-50 µL)
- Cluster tubes with caps and racks
- Pipette tips with barriers
- Powder-free nitrile gloves

**The Automated Thermal Block (Catalog No. MCH2023 [D14614252]) may be used in place of heating and cooling blocks.*

Stomacher with bags

Incubator capable of maintaining directed enrichment temperatures within ±2°C

Enrichment media (See BAX® System User Guide for details)

- Tryptic soy broth (TSB) (Formulation dependent on matrix tested. For specific details, please reference the STEC protocols in the BAX® System User Guide)
- BAX® System MP media – Catalog No. MED2003 (D12404925) (2.5 kg)

STORAGE AND SHELF LIFE

- Reagents and PCR tubes with tablets should be kept refrigerated at 2–8°C. Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- After protease has been added to the lysis buffer, shelf life of the solution is 2 weeks when stored at 2-8°C.
- 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 4ºC in a desiccation unit.

PRECAUTIONS

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

Reagents used with the BAX® System assays should pose no hazards when used as directed. Before using this product, please review the Safety Data Sheets (SDS) included with your BAX® System purchase and also available at www.hygiena.com. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

Before using this assay for the first time, install the most current version of the BAX® System software, then run a calibration report to check that "Real Time STEC Screen stx, eae", "Real Time STEC Screen stx only", "Real Time STEC Panel 1 O26, O111, O121" and "Real Time STEC Panel 2 O45, O103, O145" 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 these targets you must recalibrate the Q7 instrument to load the required dyes. Be sure to allow enough time to complete the calibration (about 1.5 to 2 hours) before starting the assay. For instructions and tips on calibrating the instrument, see the BAX® System User Guide.

ENRICHMENT PROTOCOL

1. Prepare Enrichment Broth

Prepare enrichment broth according to the manufacturer's instructions. See the BAX® System User Guide for common enrichment media recipes.

2. Collect and Enrich Samples

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- *Raw ground beef (375 g):* Homogenize sample with 1.5 L pre-warmed (46°C) glucose-containing TSB with 2mg/L novobiocin. Incubate at 41°C for 12-24 hours.
- *Raw ground beef with soy (325 g):* Homogenize sample with 975 mL pre-warmed (35°C) glucose-containing TSB with 10 g/L casamino acids and 8 mg/L novobiocin. Incubate at 41°C* for 12-24 hours.
- *Raw beef trim (325 g):* Gently massage sample with 975 mL room-temperature glucose-containing TSB with 10 g/L casamino acids and 8 mg/L novobiocin. Incubate at 41°C* for 15-24 hours.
- *Raw beef trim (375 g):* **TSB –** Gently massage sample with 1.5 L pre-warmed (46°C) glucose-containing TSB. Incubate at 41°C* for 12-24 hours. **MP Media –** Gently massage sample with 1.5 L pre-warmed (46°C) BAX® System MP media. Incubate at 41°C* for 12-24 hours.
- *Raw ground beef (25 g):* **BPW Media -** Homogenize sample with 225 mL pre-warmed (37°C) BPW. Incubate at 42°C for 10-24 hours. **mTSB Media** - Homogenize sample with 225 mL pre-warmed (37°C) mTSB + casamino acids. Incubate at 42°C for 10-24 hours
- *Flour (25 g):* Homogenize sample with 225 mL pre-warmed (42°C) mTSB with 2mg/L novobiocin. Incubate at 42°C for 24 hours.

*__Note__: Incubation temperature must be maintained between 39 ºC and 42 ºC for this assay.

TEST PROTOCOL – ALL ASSAYS

3. Prepare Equipment

3.1 Turn on the heating blocks to 37ºC and 95ºC*.
3.2 Make sure cooling blocks are chilled to 2-8ºC*.
If using the Automated Thermal Block, follow the instructions in the Thermal Block User Guide for running the Gram Negative program.
3.3 Power on the Q7 instrument and launch the BAX® System application.
3.4 Create a rack file (see User Guide for details).
IMPORTANT NOTE FOR STEC SCREENING WITH "STX ONLY": An alternative target drop-down option is available for running the "stx only" program. See the BAX® System User Guide for details.

4. Perform Lysis

4.1 Break cluster tubes apart.
4.2 Label and arrange cluster tubes in rack according to the rack file.
4.3 Prepare lysis reagent by adding 150 µL protease to one 12 mL bottle of lysis buffer.
4.4 Transfer 200 µL prepared lysis reagent to each cluster tube.
4.5 Transfer 20 µL enriched sample to the corresponding cluster tube.
4.6 Heat at 37°C for 20 minutes.
4.7 Heat at 95°C for 10 minutes.
4.8 Cool at 2-8°C for at least 5 minutes.

5. Hydrate PCR Tablets

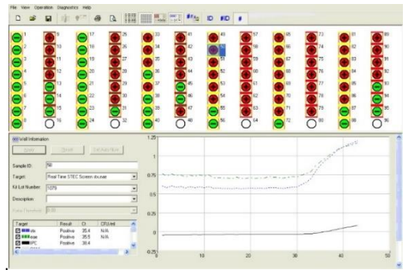
5.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.
5.2 Place a PCR tube rack onto a chilled (2-8°C) PCR cooling block.
5.3 Arrange strips of PCR tubes according to your rack file.
5.4 Remove the caps from the first strip of tubes with the decapping tool.
5.5 Transfer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

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.
***Note**: If desired, remaining lysate can be sealed and stored at 2-8°C for additional testing with the BAX® System STEC suite and/or real-time E. coli O157:H7 assay.*

7. Review Results

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



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

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

Screening Assay Results (*stx* and *eae*)

Positive result – Indicates that both *eae* and *stx* are present in that sample. The amplification plot shows a rise in the stx (blue) and eae (green) targets.**

Negative result – Indicates that the combination of *stx* and *eae* is not present in that sample. If only one of the stx or eae targets is present, the sample is considered negative.

Screening Assay Results ("STX ONLY")

Positive result – Indicates that stx is present in that sample. The amplification plot shows a rise in the stx (blue) target. The eae target is ignored. **

Negative result – Indicates that stx is not present in that sample. The eae target is ignored.

**Using the Test Protocol above, stored lysates of positive Screening samples can be run with the Panel 1 and Panel 2 assays to identify specific "Big 6" serogroup(s), if present.

Panel 1 Assay Results (*E. coli* O26, O111, O121)

Positive result – Indicates that one or more of the Panel 1 targets are present in that sample:

- *E. coli* O26 - the amplification plot shows a rise in the O26 (gold) target
- *E. coli* O111 - the amplification plot shows a rise in the O111 (grey) target
- *E. coli* O121 - the amplification plot shows a rise in the O121 (purple) target

Negative result – Indicates that none of the Panel 1 targets are present in that sample.

Panel 2 Assay Results (*E. coli* O45, O103, O145)

Positive result – Indicates that one or more of the Panel 2 targets are present in that sample:

- *E. coli* O45 - the amplification plot shows a rise in the O45 (magenta) target
- *E. coli* O103 - the amplification plot shows a rise in the O103 (brown) target
- *E. coli* O145 - the amplification plot shows a rise in the O145 (turquoise) target

Negative result – Indicates that none of the Panel 2 targets are present in that sample.

CONFIRMATION

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BAX® System results should be confirmed from the reference culture method appropriate for the sample type, such as:

- U.S. FDA Bacteriological Analytical Manual (BAM)
- USDA FSIS Microbiology Laboratory Guidebook (MLG)
- Health Canada Compendium of Analytical Methods
- International Organization for Standardization (ISO)

DISPOSAL

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

VALIDATION

The BAX® System Real-Time PCR Assays for STEC (Screening, Panel 1 and Panel 2) have been certified by the AOAC Research Institute as Performance Tested Method℠ #091301. The performance of these assays was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies for foods demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture based methods.

The USDA Food Safety and Inspection Service (USDA-FSIS) has adopted the BAX® System STEC suite for monitoring meat products and carcass and environmental sponges. See FSIS Microbiology Laboratory Guidebook (MLG) Method #5B.04 for details and protocols. Please note that the enrichment and sample preparation protocols in the MLG may differ from those in the BAX® System documentation.

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. You can also call 800-863-6842 in the U.S., 1-302-695-5300 outside the U.S., or email diagnostics.support@hygiena.com.

LIMITATION OF WARRANTY AND LIABILITY

NOTICE: READ THIS LIMITATION OF WARRANTY AND LIABILITY BEFORE USING THE BAX® SYSTEM EQUIPMENT, ASSAYS, AND/OR MEDIA ("BAX® SYSTEM"). If the terms are not acceptable, notify Hygiena immediately and arrangements will be made for return of the unused Equipment, assays, and/or media to Hygiena and for the refund of the purchase price, less shipping costs. USE OF BAX® SYSTEM EQUIPMENT, ASSAYS AND/OR MEDIA CONSTITUTES AN ACCEPTANCE OF ALL TERMS AND CONDITIONS OF THIS LIMITATION OF WARRANTY AND LIABILITY. Any additional or different terms in Buyer's purchase form(s) are material alterations and hereby rejected.

1. BAX® System Equipment should only be used with BAX® System assays.

2. When used with BAX® System assays, BAX® System Equipment is warranted 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 DEALING. User assumes all risk and liability resulting from use of the BAX® System Equipment, assays and media, whether used singly or in combination with other products.

3. BAX® Software: Hygiena warrants that for a period of 60 days from the date of first date of use by the Customer/end user, BAX® software media will be free from defect in materials and workmanship and that the BAX® software will substantially perform in accordance with the accompanying BAX® software documentation. EXCEPT FOR THE EXPRESS WARRANTY ABOVE, HYGIENA MAKES NO OTHER WARRANTY, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING. User assumes all risk and liability resulting from use of the BAX® software, whether used singly or in combination with other products.

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

5. Externally caused failures, such as improper sample preparation, improper storage or loading of reagents, electrical outages, or out-of-specification environmental conditions are not covered under this warranty. Equipment failures caused by spills, abuse, misuse, negligence, or improper operation are not covered by this warranty. Modifications, service or repairs by parties other than Hygiena-authorized providers are not covered by this warranty and, in fact, void this warranty. Circumstances beyond the reasonable control of Hygiena, including fire, explosions, accidents, flood, labor trouble or shortage, war, act of or authorized by any government, inability to obtain suitable material, Equipment, fuel, power or transportation, or acts of God are not covered under this warranty.

6. The BAX® System is designed to test only for the presence of the target organisms specified in the particular assay. The BAX® System has been tested against many, but not all, strains of the target within the sample types specified in the user documentation. Hygiena, therefore, cannot and does not make any representation or warranty that the BAX® System is capable of detecting every organism in the target genus, serotype, or species in any sample source. Accordingly, the BAX® System should not be used as the sole test for the release of user's products, nor should it be used as the sole basis for determining the safety of user's products.

7. CUSTOMER/USER ASSUMES ALL RISKS IN USING THE BAX® SYSTEM AND HYGIENA OR ITS AFFILIATES, DISTRIBUTORS, ITS LICENSORS OR REPRESENTATIVES SHALL HAVE NO LIABILITY TO CUSTOMER/USER OR TO ANY OTHER PERSON OR ENTITY FOR ANY INDIRECT, INCIDENTAL, SPECIAL, PUNITIVE, EXEMPLARY OR CONSEQUENTIAL DAMAGES WHATSOEVER, INCLUDING, BUT NOT LIMITED TO, LOSS OF REVENUE OR PROFIT, LOST OR DAMAGED DATA OR OTHER COMMERCIAL OR ECONOMIC LOSS EVEN IF CAUSED BY THE NEGLIGENCE OF HYGIENA OR ITS REPRESENTATIVES AND/OR IF HYGIENA HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND/OR IF THEY ARE FORESEEABLE.

8. THE SOLE AND EXCLUSIVE REMEDY OF CUSTOMER/USER, AND THE SOLE AND EXCLUSIVE LIABILITY OF HYGIENA, ITS AFFILIATES, DISTRIBUTORS, LICENSORS OR REPRESENTATIVES FOR ANY AND ALL CLAIMS, INCLUDING BREACH OF WARRANTY, TORT, CONTRACT, STRICT LIABILITY, NEGLIGENCE OR OTHERWISE SHALL BE LIMITED TO THE FOLLOWING: (a) Should Equipment fail to conform with the Paragraph 2 warranty, Hygiena shall, at its option: repair or replace the non-conforming Equipment with new or refurbished (repaired or rebuilt) functionally equivalent Equipment or refund the purchase price; (b) Should BAX® Software fail to conform with the Paragraph 3 warranty, Hygiena will replace it free of charge; (c) For all other claims, Hygiena may, at its option, refund the purchase price or replace the Equipment, assays or media; (d) In all cases, user is responsible for the repackaging and return of non-conforming Equipment, along with the reinstallation of new or refurbished Equipment; and (e) Equipment, assays or media shall not be returned without prior written permission from Hygiena, and then only in the manner prescribed by Hygiena. The maximum liability of Hygiena, its affiliates, distributors and licensors, and whether or not based on negligence, shall not exceed in the aggregate the amount equal to: (a) the purchase price of the BAX® System, assay or media for which damages are claimed, or (b) in the case of BAX® Software, the amount paid for the software (if licensed separately) or twenty-five thousand dollars ($25,000.00USD). Customer/user shall notify Hygiena of any claim within thirty (30) days thereof and shall commence any action against Hygiena within one (1) year of the cause of action or otherwise be barred from any remedy. Hygiena shall not be responsible for cost, loss or liability that arise from customer/user's operation of its business, and customer/user agrees to indemnify, defend and hold Hygiena and its representatives harmless from such cost, loss or liability.