DuPont™ BAX® System Real-Time PCR Assay

E. coli O157:H7

Part D14203648

KIT CONTENTS

96 PCR tubes with tablets (8x12 strips)
96 flat optical caps (8x12 strips)
1 bottle of protease (400 µL)
2 bottles of lysis buffer (12 mL)
1 package insert

INTENDED USE

Food processors and associated laboratories can use the DuPont™ BAX® System as a quick and reliable method for detecting *E. coli* O157:H7 in ground beef, beef trim, spinach and lettuce. The real-time PCR assay was designed to report yes/no results for *E. coli* O157:H7, a recognized foodborne pathogen with a low infectious dose. With a processing time of approximately 55 minutes in the BAX® System Q7 instrument, results are available in less than 11 hours for ground beef, less than 12 hours for beef trim and less than 10 hours for spinach and lettuce. 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 (see NF EN ISO 7218).

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

The BAX® System uses the Polymerase Chain Reaction (PCR) to amplify specific DNA fragments, which are stable and unaffected by growth conditions. Each fragment is a genetic sequence that is unique to the targeted organism, thus providing a highly reliable indicator that the organism is present. The BAX® System simplifies the PCR process by combining the requisite PCR reagents (primers, polymerase, nucleotides and internal positive control) into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After hydrating these tablets with prepared samples, the tubes remain sealed to reduce the potential for contamination.

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

The BAX® System PCR tablets used in this assay 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.

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MATERIALS

BAX® System Real-Time PCR Assay for *E. coli* O157:H7

BAX® System MP Media

BAX® System start-up package

- BAX® System Q7 cycler/detector
- Computer workstation with printer
- 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 (5-50µL; 20-200µL)
- Repeating pipette
- Multi-channel pipette (8 channels- 5-50µL)

Stomacher with bags

Incubator capable of maintaining 42±2°C

*Cluster tubes with caps and racks

*Tips for all pipettes

*Powder-free nitrile gloves

**Sufficient supply for 96 tests included in BAX® System start-up package*

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, if possible.

INSTRUMENT REQUIREMENTS

This assay can be used only on a BAX® System Q7 instrument running software v2.7 or higher. After installing the software but before running the real-time *E. coli* O157:H7 assay for the first time, run a calibration report to check that "Real Time *E. coli* O157:H7" appears in the list of calibration files. If it does not, you will need to 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.

STANDARD ENRICHMENT PROTOCOL

1. Prepare enrichment broth

Dissolve 22.5 g BAX® System MP media in 1 L distilled water and mix. Do not boil. Adjust pH to a final value of 7.2±0.2, then autoclave at 121°C for 15 minutes.

Note: DuPont™ StatMedia™ soluble packets may also be used to prepare BAX® System MP media. See instructions on packet or in User Guide.

2. Collect and enrich samples

2A. AOAC method

- *Ground beef*: Stomach 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.
- *Beef trim*: Mix 375 g sample by hand with 1.5 L pre-warmed (45°C) BAX® System MP media. Incubate at 42°C for 10-24 hours.
- *Spinach and lettuce*: Stomach 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.

2B. AFNOR method

- *Raw beef*: Stomach 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42±1°C for 7-24 hours.
- *Raw vegetables*: Stomach 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42±1°C for 8-24 hours.

Note: Because short enrichment times are particularly sensitive to incubation conditions, pre-warming and incubation temperatures should be strictly applied to ensure that the enrichment media reach the target temperature. Sample incubation must begin within 45 minutes of the end of media pre-warming. The use of a fan-assisted incubator is recommended.

TEST PROTOCOL

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.
3.3 Power on the Q7 instrument and launch the BAX® System application.
3.4 Create a rack file (see User Guide for details).
3.5 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

4. Perform lysis

4.1 Label and arrange cluster tubes in rack according to the rack file.
4.2 Mix lysis reagent by adding 150 µL protease to a 12-mL bottle of lysis buffer.
4.3 Transfer 200 µL lysis reagent to each cluster tube.
4.4 Transfer 20 µL enriched sample to each cluster tube.
4.5 Heat at 37°C for 20 minutes.
4.6 Heat at 95°C for 10 minutes.
4.7 Cool for 5 minutes in cooling block.

5. Hydrate PCR tablets

5.1 Place a PCR tube insert into cooling block and cover with PCR tube holder.
- For AOAC, use a chilled (2-8°C) cooling block.
- For AFNOR, use a frozen (-20°C) cooling block.

5.2 Arrange strips of PCR tubes according to your rack file.
5.3 Remove the caps from the first strip of tubes with the decapping tool.
5.4 Transfer 30 µL lysate into PCR tubes, then seal with flat optical caps.

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Note: PCR tablets must be hydrated and re-sealed within 10 minutes after removing the caps from the PCR tubes.

6. Amplify and detect

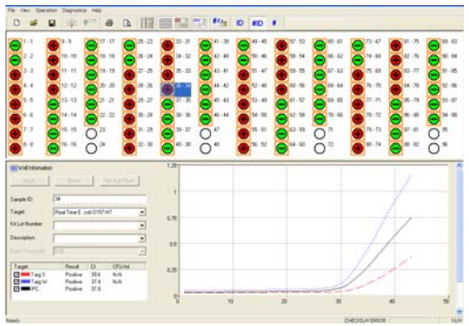
6.1 At the "Ready for Rack Load" prompt, click the NEXT button and open the instrument drawer.

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.

7. Review results

Qualitative results are displayed as a grid of well icons in the top half of the screen:



- If both *E. coli* O157:H7 targets are present in the sample, the well is red with a "plus" sign (positive).
- If either of the *E. coli* O157:H7 targets is not present in the sample and the internal positive control (IPC) is positive, the well is green with a "minus" sign (negative).
- If both *E. coli* O157:H7 targets and the internal positive control (IPC) are negative, the well is yellow with a "question mark" sign (indeterminate).
- A well that is yellow and outlined in red with a red bar across the center indicates signal error. Call DuPont for assistance.

CONFIRMATION

AOAC Method

If desired, ground beef and trim samples can be confirmed according to the USDA-FSIS standards by performing an IMS step and streaking presumptive *E. coli* O157:H7 cells onto Rainbow® Agar O157 (Biolog Inc., Hayward, California). In addition, directly streak 1 uL enrichment onto Rainbow® Agar O157 without IMS to confirm rough *E. coli* O157:H7 strains which may not be captured by IMS. Incubate all plates for 18-24 hours at 37°C.

Spinach and lettuce samples can be confirmed according to FDA standards by streaking 10 uL enrichment onto CT-SMAC plates and incubating for 18-24 hours at 35-37°C. In addition, for samples with high levels of background flora, perform an IMS step before streaking presumptive *E. coli* O157:H7 cells onto CT-SMAC plates.

For all confirmation procedures, typical isolates should be confirmed with appropriate biochemical and serological methods.

Method-Certified AFNOR Validation

Presumptive positives must be confirmed by one of the following methods:

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. See NF EN ISO 7218 for the confirmation protocol of standardized methods.
2. Streak 50 uL enrichment onto CT-SMAC plates and incubate for 18-24 hours at 35-37°C. Check plates for typical *E. coli* O157:H7 colonies. If no typical colonies appear on CT-SMAC plates, follow the confirmation protocol for *E. coli* O157:H7 described in technical bulletin 23C-013-0804 to recover *E. coli* O157:H7 with an IMS step. In some cases, a second IMS step may be necessary.

Typical isolates should be confirmed with appropriate latex tests (O157 and H7 latex tests) on characteristic colonies (1-5 colonies if the first isolate is not confirmed as *E. coli* O157:H7). For example, Wellcollex latex test for *E. coli* O157:H7 (Oxoid reference R30959601) can be used. In the event of discordant results, laboratories must ensure the validity of reported results.

DISPOSAL

Decontaminate materials and dispose of biohazardous waste according to your site practices and as required by federal, state and local regulations. If you have questions about proper waste disposal at your site for the materials provided by DuPont, please call for assistance.

PRECAUTIONS

The BAX® System method includes sample prep 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 Material Safety Data Sheets (MSDS) included with your BAX® System purchase and also available at www.fooddiagnostics.dupont.com. Refer to your site practices for safe handling of materials at extreme temperatures.

VALIDATION

AOAC Method

The BAX® System Real-Time PCR Assay for *E. coli* O157:H7 has been certified by the AOAC Research Institute as Performance Tested Method℠ #031002, with sensitivity and specificity equivalent to the official USDA-FSIS and FDA-BAM culture-based methods. AOAC-RI validation studies were performed on ground beef, beef trim, spinach and green lettuce. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications.

Method-Certified AFNOR Validation

The BAX® System Real-Time PCR Assay for *E. coli* O157:H7 has been certified on the BAX® System Q7 instrument with software v2.7 according to AFNOR validation rules (#QUA 18/07 – 07/10). Validation studies conducted according to ISO 16140 standards found this test kit's performance to satisfy the AFNOR certification rules for raw beef and vegetables. For more information including validity dates, see Certificate #QUA 18/07 – 07/10 available at www.afnor-validation.com.

Sample sizes larger than 25g have not been tested in the framework of the AFNOR validation mark.

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. In the U.S., you can call 800-863-6842, fax 302-351-6454, or email diagnostics.support@dupont.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 DuPont immediately and arrangements will be made for return of the unused Equipment, assays, and/or media to DuPont 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. DuPont 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.

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4. The accuracy of the BAX® System can be affected by factors over which DuPont 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 DuPont. Because of the large number of factors over which DuPont has no control, DuPont makes no promise or guarantee of the accuracy of or results obtained from the use of the BAX® System. In particular, DuPont 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 DuPont-authorized providers are not covered by this warranty and, in fact, void this warranty. Circumstances beyond the reasonable control of DuPont, 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. DuPont, 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 DUPONT 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 DUPONT OR ITS REPRESENTATIVES AND/OR IF DUPONT HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND/OR IF THEY ARE FORESEEABLE.

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