DuPont™ BAX® System Real-Time PCR Assay

Salmonella

Part D14306040

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 081201

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BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

QUA 18/08 – 03/15
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org

KIT CONTENTS

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

The test protocol in this package insert has been revised to specify parameters for hydrating PCR tablets. Please read these instructions carefully before using this assay.

INTENDED USE

Food processors and associated laboratories can use the DuPont™ BAX® System as a quick and reliable method for detecting *Salmonella* in a variety of food and environmental samples. This real-time PCR assay was designed to report yes/no results for *Salmonella* after approximately one hour of processing in the BAX® System Q7 instrument. Results are available in less than 24 hours for most sample types.

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).

PRINCIPLE OF THE METHOD

The BAX® System is a DNA-based molecular detection method that combines automated Polymerase Chain Reaction (PCR) technology with automated detection to identifying foodborne pathogens, spoilage organisms and other microbes in raw ingredients, finished products and environmental samples. See the BAX® System User Guide for an overview of the BAX® System method and PCR.

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.

MATERIALS

BAX® System Real-Time PCR Assay for *Salmonella*

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

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±2ºC
- 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 35±2°C and/or 42±2°C

Note: Health Canada and AFNOR Certification standards require an incubator capable of maintaining ±1°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.

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 assay, 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.

INSTRUMENT REQUIREMENTS

This assay can be used only on a BAX® System Q7 instrument running software v3.1 or higher. 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 *Salmonella*" 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 *Salmonella*", 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.

STANDARD 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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Follow the enrichment protocols for the selected sample types as described in the most current version of the BAX® System User Guide.

Method Approved by AFNOR Certification

User should comply with Good Laboratory Practices (refer to ISO 7218) and follow the general requirements described in ISO 22174:2005 when using PCR methods. For preparation of initial suspensions, follow instructions of EN ISO 6579 and EN ISO 6887 standards. In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.

- Raw beef (short protocol) – Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 41.5±1°C for 10-24 hours.
- Meat products (including meat with spices or herbs), seafood, vegetables and pet food – Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37±1°C for 16-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37±1°C for 3-4 hours.
- Egg products – Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37±1°C for 18-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37±1°C for 3-4 hours.

Note: Due to the sensitivity of short enrichment protocols, it is important that incubation times and temperatures are followed as closely as possible. Verify that media is sufficiently pre-warmed before adding samples, and that the delay between pre-warming media and adding samples does not exceed 45 minutes. Use of a ventilated incubator during the incubation 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).

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 5 µL enriched sample to each cluster tube.

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

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4.5 Heat at 37°C for 20 minutes.

4.6 Heat at 95°C for 10 minutes.

4.7 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 insert onto a chilled (2-8°C) cooling block and cover with PCR tube holder.

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. Transfer 30 µL lysate into PCR tubes, then seal with flat optical caps.

5.5 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.
Note: PCR tablets must be hydrated and re-sealed within 10 minutes after removing the caps from the PCR tubes.

5.6 Let PCR tubes sit in the cooling block for 10-30 minutes before loading 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 well icons in the top half of the screen:

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

CONFIRMATION

Method Approved by AOAC

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

Note: For confirmation methods that require an additional plating media of choice, Oxoid Brilliance™ Salmonella plates (Oxoid PO5098A or Oxoid CM1092 & SR 0194) are recommended.

Method Approved by AFNOR Certification

All samples identified as positive by the BAX® System method must be confirmed as follows:

- Using the conventional tests described in the methods standardized by CEN or ISO from colonies (including the purification step). The confirmation step must start from the BPW (ISO) enrichment.
- For raw beef (short protocol) and other raw meat products - Transfer 10 µL BPW enrichment to RVS broth and incubate 41.5±1°C for 21-27 hours. Streak 10 µL of the RVS enrichment to Brilliance Salmonella Agar and incubate at 3 ±1°C for 21-27 hours; streak an additional 10 µL RVS enrichment to XLD Agar and incubate at 37±1°C for 18-24 hours. Confirm presumptive positive colonies with a latex test.
- For egg products, seafood, vegetables and pet food – Streak 10 µL BPW enrichment to Brilliance Salmonella Agar and incubate at 37±1°C for 21-27 hours; streak an additional 10 µL BPW enrichment to XLD Agar and incubate 37±1°C for 18-24 hours. Confirm presumptive positive colonies with a latex test.

In the event of discordant results (positive with the alternative method, non-confirmed by one of the means described above, and in particular for the latex test) the laboratory must follow the necessary steps to ensure the validity of the result obtained. Some strains of Salmonella belonging to the serovar Dublin, may show weak magenta pigmentation, because of their low esterase activity.

BPW enrichments may be stored at 2-8°C for up to 72 hours after enrichment to allow for confirmation of PCR positive results and in case of a need to repeat the PCR analysis.

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.

VALIDATION

The BAX® System Real-Time PCR Assay for *Salmonella* has been certified by the AOAC Research Institute as Performance Tested Method℠ #081201, 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, chicken carcass rinses, cream cheese, fresh bagged lettuce, dry pet food, and environmental sponges. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications.

The BAX® System Real-Time PCR Assay for *Salmonella* has been certified as an AOAC INTERNATIONAL Official Method of Analysis (OMA) #2013.02 for detecting *Salmonella* in a variety of foods and environmental surfaces. Validation studies were performed on raw ground beef, beef trim, frankfurters, shrimp, ground turkey, chicken wings, poultry rinses, whole powdered dried eggs, shell eggs, fresh bagged lettuce, frozen peas, orange juice, cream cheese, non-fat dry milk, ice cream, peanut butter, cocoa, white pepper, milk-based infant formula, dry pet food, and on stainless steel, ceramic tile and plastic surfaces.

The BAX® System Real-Time PCR Assay for *Salmonella* has been certified as #QUA 18/08-03/15 according to NF VALIDATION rules. Validation studies conducted according to ISO 16140 standards found this test kit's performance to satisfy the ISO 16140 standard and NF VALIDATION rules for meat products, egg products, seafood, vegetables and pet food. For more information about the end of validity of the NF VALIDATION certification, please refer to certificate QUA 18/08-03/15 available at http://nf-validation.afnor.org.

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.

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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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