DuPont™ BAX® System PCR Assay for *Salmonella* 2

Part D14368501

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 *Salmonella* in a variety of foods and environmental surfaces. The *Salmonella* 2 PCR assay performs just like the original *Salmonella* assay, providing yes/no results for a wide range of *Salmonella* species. The difference is proprietary "hot-start" technology in the *Salmonella* 2 PCR tablets, which keeps the reaction enzyme inactive until PCR begins. This improvement greatly reduces the opportunity for non-specific PCR product to form and improves the specificity of the assay while requiring no change to the protocol.

As with the original *Salmonella* assay, the BAX® System *Salmonella* 2 assay can detect concentrations as low as 10^4 cfu/ml, after enrichment. Results are available next-day with approximately 3.5 hours processing time in the BAX® System instrument. 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

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.

MATERIALS

BAX® System PCR Assay for *Salmonella* 2 (D14368501)

BAX® System start-up package

- BAX® System cycler/detector
- Computer workstation with printer
- Heating blocks with inserts capable of maintaining 37±2°C and 95±2°C
- 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

Enrichment media (see User Guide for recommended enrichments)

*Cluster tubes with caps and racks

*Tips for all pipettes

*Powder-free nitrile gloves

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

STORAGE AND SHELF LIFE

Reagent packages 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.

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.

TEST PROTOCOL FOR ENRICHED SAMPLES

1. Collect and enrich samples

Samples should be enriched according to your standard laboratory enrichment protocols. For a list of recommended enrichment protocols for your sample type, see the *Salmonella* enrichments described in the BAX® System User Guide.

In the context of NF VALIDATION, follow instructions of EN ISO 6579 and EN ISO 6887 standards for preparation of initial suspensions.

2. Prepare equipment

2.1 Turn on the heating blocks and set the temperatures for 37°C and 95°C.
2.2 Make sure cooling blocks are chilled to 2-8°C.
2.3 Power on the BAX® System instrument and launch the BAX® System application.
2.4 Create a rack file (see User Guide for details).
2.5 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

3. Perform lysis

3.1 Label and arrange cluster tubes in rack according to the rack file.
3.2 Mix lysis reagent by adding 150 µL protease to a 12-mL bottle of lysis buffer.
3.3 Transfer 200 µL lysis reagent to each cluster tube.
3.4 Transfer 5 µL enriched sample to each cluster tube.
3.5 After all transfers have been completed, secure the caps.
3.6 Heat at 37°C for 20 minutes.
3.7 Heat at 95°C for 10 minutes.
3.8 Cool for 5 minutes in cooling block.

Note: You must finish using the cooling blocks within 30 minutes of removing them from the refrigerator to keep samples at the correct cooling temperature.

4. Hydrate PCR tablets

IMPORTANT NOTE: Be sure the instrument has reached the correct load temperature and prompts you to continue before beginning to hydrate tablet.

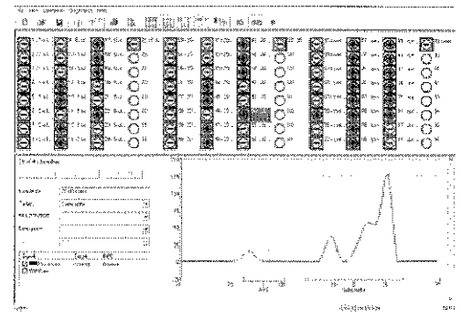
4.1 Place a chilled PCR tube insert into cooling block and cover with PCR tube holder.
4.2 Arrange strips of PCR tubes according to your rack file.
4.3 Transfer 50 µL lysate to the first strip of PCR tubes, then seal with flat optical caps. Repeat until all PCR tubes have been re-sealed.

5. Amplify and detect

5.1 At the "Ready for Rack Load" prompt, click the NEXT button and open the instrument drawer.
5.2 Place the rack of PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.
5.3 Close the drawer, and click the NEXT button to begin automated processing.

6. Review results

When processing is complete (about 3.5 hours), follow the screen prompts to remove your samples and review the results. Results are displayed as a grid of well icons in the top half of the screen:



 Rev 21C-014.3-0814 A01347563

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, results for sample types regulated by the FDA can be confirmed by streaking 10 µL enrichment onto BS agar, XLD agar and HE agar plates. Incubate all plates for 22-26 hours at 35°C, then examine for typical *Salmonella* colonies. Confirm all suspect colonies using the biochemical and serological methods described in the FDA-BAM.

Results for sample types regulated by the USDA can be confirmed by streaking 10 µL enrichment onto DMLIA or XLT4 agar and BGS agar plates. Incubate all plates for 24-48 hours at 35°C, then examine for typical *Salmonella* colonies. Confirm all suspect colonies using the biochemical and serological methods described in the USDA-FSIS MLG.

Method approved by AFNOR Certification

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

1. Follow one of the conventional testing methods described by CEN or ISO, including purification. The laboratory must comply with good laboratory practice (refer to EN ISO 7218 standard).
2. Isolate *Salmonella* colonies on a selective plate from the last enrichment media as described the EN ISO 6579:2002 reference method.
3. For raw meats enriched with BAX® System MP media, transfer MP media into RVS tube, followed by isolation on Brilliance™ *Salmonella* (Oxoid PO5098A) and confirmation of typical colonies by a latex test.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

Confirmation should be completed within 24 hours of regrowth in BHI. Typical isolates should be confirmed with appropriate biochemical tests on characteristic colonies (1-5 colonies if the first isolate is not confirmed as *Salmonella*).

For food matrices with a high level of background flora, if no suspect colonies are isolated directly from the direct plating described above, transfer the last enrichment to RVS broth, incubate at 41.5°C for 24 hours, and isolate on selective agar plates according to the ISO 6579:2002 reference method.

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 PCR Assay for *Salmonella* 2 has been certified by the AOAC Research Institute as a method extension of Performance Tested℠ Method #100201. This test kit's performance was reviewed by AOAC-RI and found to perform to the manufacturer's specifications. Validation studies on a variety of foods demonstrated BAX® System sensitivity and specificity equal to or better than the official FDA-BAM or USDA-FSIS culture-based methods.

The BAX® System PCR Assay for *Salmonella* 2 has been certified by AOAC International as a method extension of Official Method of Analysis℠ 2003.09. Validation studies on frankfurters, ground beef, cream cheese and dry pet food demonstrated BAX® System sensitivity and specificity equal to or better than the official FDA-BAM or USDA-FSIS culture-based methods.

The BAX® System PCR Assay for *Salmonella* 2 has been approved by AFNOR Certification as a method modification for #QUA 18/03 – 11/02. The NF VALIDATION studies conducted according to EN ISO 16140 standards found this test kit's performance to satisfy the NF VALIDATION rules for all human food products, animal feed and environmental samples. For more information, visit www.afnor-validation.com.

Test portions weighing more than 25 g have not been tested in the context of NF VALIDATION.

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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