

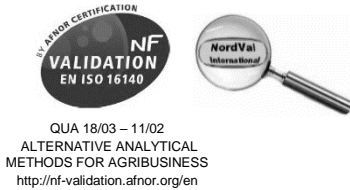


BAX® System PCR Assay for

Salmonella Part KIT2012 (D11000133)



Salmonella 2 Part KIT2011 (D14368501)



Kit Contents

- 96 PCR tubes with tablets (1 bag of 12 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 as a quick and reliable method for detecting *Salmonella* in a variety of foods and environmental surfaces. Both PCR assay's (*Salmonella* and *Salmonella 2*) were designed to report yes/no results for *Salmonella* spp. at concentrations as low as 10⁴ cfu/mL after enrichment. The difference in the *Salmonella 2* PCR assay is a proprietary "hot-start" technology in the 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.

With a processing time of approximately 3.5 hours 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. The laboratory must comply with good laboratory practice (see ISO 7218 standard).

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, Polymerase Chain Reaction (PCR) technology.

MATERIALS

- BAX® System PCR Assay for *Salmonella* (Part KIT2012 [D11000133])
- BAX® System PCR Assay for *Salmonella 2* (Part KIT2011 [D14368501])

BAX® System start-up package (equipment and supplies for up to 192 tests)

- BAX® System cycler/detector and computer work station
- Heating blocks with inserts* capable of maintaining 37±2°C and 95±3°C
- 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

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

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

Note: For an NF-Validation method, please note that for the preparations of master solutions, you must follow the instructions from the EN ISO 6887 standards.

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 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 "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 "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.

ENRICHMENT PROTOCOL

1. Prepare Enrichment Broth

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

2. Collect and Enrich Samples

Method Approved by AOAC

ENRICHMENT PROTOCOL - STANDARD MEDIA

- **Meat and poultry:** Homogenize (if ground or processed) or gently massage (if not ground) 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours.
- **Mozzarella cheese:** Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Liquid Eggs:** Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Peanut Butter:** Homogenize 25 g sample with 225 mL LB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Alfalfa Sprouts:** Homogenize 25 g sample with 225 mL pre-warmed (42°C) BPW with 20 mg/L novobiocin. Incubate at 42°C for 20-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Raw Frozen Fish:** Homogenize 25 g sample with 225 mL LB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours.
- **Orange Juice:** Homogenize 25 g sample with 225 mL Universal Pre-enrichment broth. Let stand at room temperature 55-65 minutes. Do not adjust pH. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Milk Chocolate:** Homogenize 25 g sample with 225 mL reconstituted nonfat dry milk. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Add 0.45 mL 1% brilliant green dye solution. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Nonfat dry milk:** Pour 25 g sample slowly over 225 mL Brilliant Green Water (2 mL 1% brilliant green dye solution/L deionized water). Let stand at room temperature 55-65 minutes. Do not mix or adjust pH. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

- **Black Pepper:** Homogenize 25 g sample with 225 mL TSB. Let stand at room temperature 55-65 minutes. Adjust pH to 6.8±0.2. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Custard, 2% milk, chilled ready meal, cooked fish, prawns, macaroni, pizza dough, frozen peas and dry pet food:** Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Soy protein flour:** Pour 25 g sample slowly over 225 LB in a flask. Let stand for 55-65 minutes at room temperature. Cap loosely. Incubate at 37°C for 18-22 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- **Environmental sponges:** Sample a 4 x 4 in (10 x 10 cm) environmental area with a sponge pre-moistened with 10 mL D/E Neutralizing Broth or equivalent.

Finished Product Areas – Homogenize sponge with 225 mL pre-warmed (35°C) LB. Incubate at 35°C for 22-26 hours.

Raw Materials Areas – Homogenize sponge with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

ENRICHMENT PROTOCOL - BAX® SYSTEM MP MEDIA

- **Ground beef:** Homogenize 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.
- **Beef trim:** Gently massage 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 8-24 hours.
- **Spinach and Lettuce:** Combine 25 g sample with 225 mL pre-warmed (42°C) BAX® System MP media and swirl to soak entire sample. Incubate at 42°C for 8-24 hours.

Method Approved by AFNOR Certification and NordVal

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

For preparation of initial suspensions, follow instructions of EN ISO 6579 and EN ISO 6887 standards.

- **Raw meats and poultry (without spices):** Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37°C for 16-20 hours.

- **Dairy (except dried powdered milk):** Homogenize 25 g sample with 225 mL BPW supplemented with 20 mg/L novobiocin. Incubate at 42°C for 20-24 hours.
- **Raw beef (including seasoned and frozen)** in MP media: Homogenize 25 g sample in 225 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.
- **Other raw meat (including seasoned and frozen)** in MP media: Homogenize 25 g sample in 225 mL BAX® System MP media. Incubate at 42°C for 24 hours.
- **All other foods and environmental samples:** Homogenize 25 g sample with 225 mL BPW. Incubate at 37°C for 16-20 hours. Transfer 10 µL enriched sample to 500 µL room temperature BHI. Incubate at 37°C for 3-4 hours.

Note: Due to the sensitivity of short enrichment times 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 is recommended.

TEST PROTOCOL

3. Prepare Equipment

- 3.1 Turn on the heating blocks for 37°C and 95°C*.
- 3.2 Make sure cooling blocks are chilled to 2-8°C. **If using the Automated Thermal Block, follow the instructions in the Automated 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).

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 lysis reagent to each cluster tube.
- 4.5 Transfer 5 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 5 minutes.

5. Hydrate PCR Tablets

- 5.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

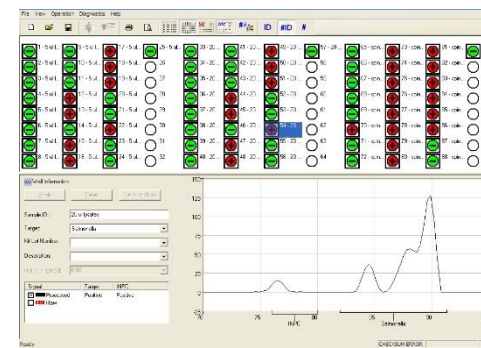
- 5.2 Place a PCR tube rack onto a chilled (2-8°C) PCR cooling block.
- 5.3 Arrange strips of PCR tubes according to your rack file.
- 5.4 Remove the caps from the first strip of tubes with the decapping tool.
- 5.5 Transfer 50 µL lysate (from step 4.8) into PCR tubes, then seal with flat optical caps.
- 5.6 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

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-coded 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.

CONFIRMATION

Method Approved by AOAC

If desired, BAX® System results can 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)

Method Approved by AFNOR Certification and NordVal

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

- Using the conventional testing methods described by CEN or ISO, including purification from the last enrichment broth, if required.
- Direct plating on selective media (as described in the ISO 6579 reference method) from the enrichment medium of the BAX® method.
- For raw meats enriched with BAX® System MP media, transfer 100 µL MP enrichment to RVS broth and incubate at 41.5°C for 21-27 hours. Streak 10 µL of the RVS enrichment to Brilliance™ Salmonella Agar and incubate at 37°C for 21-27 hours. Confirm typical colonies with Thermo Scientific™ Oxoid™ Salmonella Test Kit.

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.

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.

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 PCR Assay for *Salmonella* and the BAX® System PCR Assay for *Salmonella* 2 have been certified by the AOAC Research Institute as Performance TestedSM Method #100201. This test kit's performance was reviewed by AOAC-RI and found to perform to the manufacturer's specifications. Validation studies for foods and surfaces demonstrated BAX® System sensitivity and

specificity equal to or better than the reference culture-based methods.

The BAX® System PCR Assay for *Salmonella* and the BAX® System PCR Assay for *Salmonella 2* have been certified by AOAC International as Official Method of AnalysisSM (OMA) #2003.09. Validation studies for the BAX® System PCR Assay for *Salmonella* were performed on raw ground beef, raw ground chicken, raw frozen fish, mozzarella cheese, frankfurters and orange juice. Validation studies for the BAX® System PCR Assay for *Salmonella 2* were performed on frankfurters, raw ground beef, cream cheese and dry pet food.

The BAX® System PCR Assay for *Salmonella* and BAX® System PCR Assay for *Salmonella 2* have been certified as #QUA 18/03-11/02 according to NF VALIDATION rules. Validation studies conducted according to ISO 16140-2 standards found this test kit's performance to satisfy the NF VALIDATION rules for all human food products, by conducting validation assays on a broad range of food and animal feed and production environmental samples (excluding primary production samples). For more information, including validity dates, please refer to certificate QUA 18/03-11/02 available at <http://nf-validation.afnor.org>.

The software version approved in the scope of NF-Validation certification is disclosed in the certificate. For more information about the end of validity of the NF-Validation certification, please refer to the certificate available on the website or upon request to Hygiena representative.

The BAX® System PCR Assay for *Salmonella* and BAX® System PCR Assay for *Salmonella 2* have been certified by NordVal International as fulfilling the requirements of the NordVal Validation Protocol (NordVal #030). This test kit's performance was reviewed by NordVal International and was found to perform to the manufacturer's specifications for food, feed and environmental samples. Validation studies demonstrated BAX® System sensitivity and specificity better than or equal to the reference culture-based methods.

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 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 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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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.

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