hygiena

BAX® System X5

PCR Assay for *Salmonella*

Part KIT2025 (D15407187)

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 100201

KIT CONTENTS

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

NF VALIDATION EN ISO 16140
QUA 18/03 – 11/02
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

INTENDED USE

Food processors and associated laboratories can use the BAX® System X5 as a quick and reliable method for detecting *Salmonella* species from a variety of food and environmental surfaces. This assay is designed to report yes/no results for *Salmonella* at concentrations as low as 10^4 cfu/mL after enrichment. With a processing time of approximately 3.5 hours in the BAX® System X5 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 X5 User Guide for an overview of how the BAX® System method uses automated, Polymerase Chain Reaction (PCR) technology.

MATERIALS

BAX® System X5 PCR Assay for *Salmonella* (Part KIT2025 [D15407187])

BAX® System X5 start-up package (equipment and supplies)

- BAX® System X5 instrument and associated computer
- Automated Thermal Block*
- 32-well aluminum cold block with insulator
- 32-well PCR tube holder
- Capping/decapping tools
- Adjustable pipettes (5-50 µL; 20-200 µL)
- Repeating pipette
- Multi-channel pipette (8 channels – 5-50 µL)
- Cluster tubes with caps and racks
- Pipettor tips with barriers
- Powder free nitrile gloves

**Analog heating and cooling blocks may be used in place of the Automated Thermal Block. See the BAX® System X5 User Guide for details.*

Stomacher with bags

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

Enrichment media (See BAX® System X5 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

- Reagents 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.
- 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.
- 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 X5 PCR 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 X5 purchase and also available at www.hygiena.com. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

The BAX® System X5 PCR Assay for *Salmonella* is designed for use only with the BAX® System X5 instrument. Do not use this assay with the BAX® System Q7 instrument or other PCR platforms.

BAX® System X5 PCR assays should always be run using the most current BAX® System X5 software version for optimal performance.

ENRICHMENT PROTOCOL – STANDARD MEDIA

1. Prepare Enrichment Broth

Prepare standard enrichment broth according to the manufacturer's instructions.

2. Collect and Enrich Samples
Method Approved by AOAC

- *Frankfurters, chipped ham and cooked chicken*– Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.
- *Raw ground beef* – For BPW- Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. For mTSB- Homogenize 25 g sample with 75 mL room temperature mTSB. Incubate at 42°C for 20-24 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.
- *Milk Chocolate* – Homogenize 25 g sample with 225 mL reconstituted nonfat dry milk with 0.45 mL 1% brilliant green dye solution per liter. Let stand at room temperature for 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.
- *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 for 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 for 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.
- *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.
- *Orange juice* – Homogenize 25ml in 225 mL of Universal Pre-enrichment Broth. Let stand at room temperature for 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.
- *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.

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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 prewarmed 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.*

ENRICHMENT PROTOCOL – BAX® SYSTEM MP MEDIA

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 at 25°C, then autoclave at 121°C for 15 minutes.

2. Collect and Enrich Samples
Method Approved by AOAC

- *Ground Beef (65 g)* – Homogenize 65 g sample with 585 mL pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.
- *Beef Trim (375 g)* – Gently massage 375 g sample with 1.5 L pre-warmed (45°C) BAX® System MP media. Incubate at 42°C for 9-24 hours.
- *Spinach and Lettuce (25 g)* – 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.

TEST PROTOCOL

3. Prepare Lysis Reagent

To create a full bottle of BAX® System lysis reagent (prepares about 60 samples):

3.1. Add 150 µL protease to one 12 mL bottle of lysis buffer.

To create smaller volumes of BAX® System lysis reagent (prepares about 4 samples):

3.2. Add 12.5 µL protease to 1 mL of lysis buffer in a separate sterile container.

4. Perform Lysis

4.1 Open the BAX® System X5 software and create a rack file (See BAX® System X5 User Guide for details).

4.2 Turn on the Automated Thermal Block and select the Gram Negative program.
***Note**: Lysis may also be performed using separate analog heating and cooling blocks. See the BAX® System X5 User Guide for details and protocols.*

4.3 Break cluster tubes apart.

4.4 Label and arrange cluster tubes in rack according to the rack file.

4.5 Transfer 200 µL prepared lysis reagent to each cluster tube.

4.6 Transfer 5 µL enriched sample to the corresponding cluster tube.

4.7 After all transfers have been completed, secure the caps.

4.8 At the "Load Samples" prompt on the Automated Thermal Block, place the samples in the cluster tube rack onto the Automated Thermal Block.

4.9 Press the SELECT/CONTINUE button to begin automated lysis.

5. Hydrate PCR Tablets

5.1 Place a 32-well PCR tube holder onto a chilled (2-8°C) PCR cooling block.

5.2 Arrange strips of PCR tubes according to your rack file.

5.3 Remove the aluminum block with the cluster tubes of sample lysate from the Automated Thermal Block.

5.4 Press the SELECT/CONTINUE button on the Automated Thermal Block to complete the program.

5.5 Remove the caps from the first strip of tubes with the decapping tool.

5.6 Transfer 50 µL lysate (from step 5.3) into PCR tubes, then seal with flat optical caps.

5.7 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

6. Complete a BAX® System Process Run

6.1 From the Info Tab labeled "Instrument" in the BAX® System X5 software window, click the RUN button to begin pre-heating the instrument.

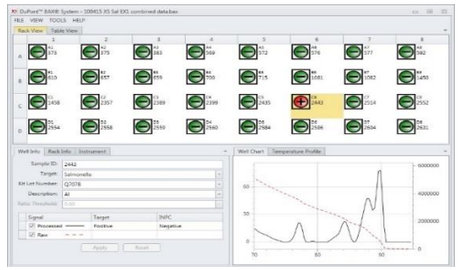
6.2 Remove the PCR tube holder with PCR tubes from the cooling block and **visually check PCR tubes to ensure the liquid contains no air bubbles and tubes are clean.**
IMPORTANT: If air bubbles are present in the liquid, gently tap or flick the tubes to allow all air bubbles to escape.
If the outside of the PCR tubes have dust, oils or residue, clean the outside of each PCR tube with a clean, lint-free lab wipe.

6.3 At the "Ready to load samples" prompt, open the instrument lid and load the PCR tubes into the instrument mount according to your rack file.
IMPORTANT: To process fewer than 32 samples, load samples first in Row A and Row D, starting at the corners. Once the two outer rows are full, then use Row B and Row C to load samples in a symmetric pattern to ensure the instrument mount remains balanced.

6.4 Close the instrument lid as soon as possible to prevent the instrument from cooling. The process run begins automatically.

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

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

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.
- 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 a latex test.
- For all categories but raw meat products, streak 10 µL of enrichment (BPW/BHI) to Brilliance *Salmonella* Agar and incubate at 37°C for 21-27 hours; streak an additional 10 µL BPW enrichment to XLD agar and incubate at 37°C for 18-24 hours. Perform appropriate latex, biochemical and/or serological tests to confirm typical *Salmonella* colonies.

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 according to your site practices and as required by federal, state and local regulations.

VALIDATION

The BAX® System X5 PCR Assay for *Salmonella* has been certified by the AOAC Research Institute as 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 for foods and environmental surfaces demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture-based methods.

The BAX® System X5 PCR Assay for *Salmonella* has 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.

TECHNICAL ASSISTANCE

For questions or comments, please contact your Hygiena representative or Diagnostics Support at 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 X5 Equipment should only be used with BAX® System X5 assays.

2. When used with BAX® System X5 PCR assays, BAX® System X5 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 X5 PCR 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 X5 Equipment, PCR assays and media, whether used singly or in combination with other products.

3. BAX® System software: Hygiena warrants that for a period of 60 days from the date of first date of use by the Customer/end user, BAX® System software media will be free from defect in materials and workmanship and that the BAX® System software will substantially perform in accordance with the accompanying BAX® System 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® System 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® System 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 X5 Equipment, PCR assays or media for which damages are claimed, or (b) in the case of BAX® System 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.

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