



BAX® System Real-Time PCR Assay

Salmonella

Product Number KIT2006



Kit Contents

- 96 PCR tubes with tablets (2 bags of 6 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, hemp and cannabis flowers, and environmental surfaces. The real-time PCR assay was 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 75 minutes in the BAX System Q7 instrument, the method returns results comparable to culture methods, but with a significantly faster time to result.

With the SalQuant® method application, the intended use can be expanded to include quantification of various poultry, beef and pork matrices. In addition, the Real-Time PCR assay can also be used as an alternative to the traditional MPN method for raw comminuted turkey and chicken, whole carcass poultry rinses, and raw beef trim.

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). For an NF-validation method, the laboratory must follow ISO 22174 (Microbiology of food and animal feeding stuffs-Polymerase chain reaction (PCR) for the detection of food-borne pathogens--General requirements and definitions).

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 US or non-US 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 Q7 User Guide for an overview of how the BAX System method uses automated, real-time Polymerase Chain Reaction (PCR) technology.



MATERIALS

BAX System Real-Time PCR Assay for *Salmonella* (Product Number KIT2006)

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

- BAX System Q7 cycler/detector and computer workstation
- 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 pipettors (5-50 µL; 20-200 µL)
- Repeating pipettor
- Multi-channel pipettor (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) can 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 preparation 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 to 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, the shelf life of the solution is 2 weeks when stored at 2 to 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 4 °C 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 product, 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 “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.

ENRICHMENT PROTOCOL - STANDARD ENRICHMENT MEDIA

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

Method Approved by AOAC

- ***Ground beef:***
 - **For 25 g:** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BPW. Incubate at 35 °C for 20-24 hours.
 - **For 375 g:** Homogenize 375 g sample with 1.5 L pre-warmed (45 to 46 °C) mTSB with 2 mg/L novobiocin. Incubate at 39 to 42 °C for 22-26 hours.
- ***Ground beef with soy:***
 - **For 25 g:** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BPW. Incubate at 35 °C for 8-24 hours.
 - **For 325 g:** Homogenize 325 g sample with 975 mL pre-warmed (35 °C) mTSB with 10 g/L casamino acids and 8 mg/L novobiocin. Incubate at 35 °C for 20-24 hours.
- ***Beef trim:***
 - **For 25 g:** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BPW. Incubate at 35 °C for 20-24 hours.
 - **For 325 g:** Homogenize 325 g sample with 1.5 L pre-warmed (41 °C) BAX System MP media. Incubate at 39 to 42 °C for 16-24 hours.
- ***Poultry rinses:*** Homogenize 30 mL BPW sample rinsate with 30 mL pre-warmed (35 °C) BPW. Incubate at 35 °C for 22-26 hours.
- ***Ground turkey and chicken wings:*** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BPW. Incubate at 35 °C for 16-24 hours.
- ***Frankfurters:*** Homogenize 325 g sample with 1400 mL pre-warmed (35 °C) BPW. Add additional BPW to reach a total media volume of 2925 mL. Incubate at 35 °C for 18-24 hours.
- ***Shrimp:*** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35 °C for 22-26 hours.
- ***Shell eggs:*** Combine 20 eggs (~1000 mL) into a sterile container with 2 L pre-warmed (42 °C) BAX System MP media. Incubate at 42 °C for 48 hours. *Optional* – Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.



- *Eggs, Dried:*
 - **For LB Enrichment** – Add approximately 15 mL pre-warmed (35 °C) LB to 25 g sample and stir to smooth. Add 3 additional aliquots of LB of 10 mL, 10 mL, and 190 mL (total media volume 225 mL), stirring after each addition. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 . Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
 - **For BPW Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BPW. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- *Peanut butter:*
 - **For LB Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 , if necessary. 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.
 - **For BPW Enrichment** – 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.
- *Lettuce:*
 - **For MP Media Enrichment** – Add 25 g sample to 225 mL pre-warmed (35 °C) BAX System MP media and swirl 25 times clockwise and 25 times counterclockwise to mix. Incubate at 35 °C for 10-24 hours.
 - **For LB Enrichment** – Add 25 g sample to 225 mL pre-warmed (35 °C) LB and swirl 25 times clockwise and 25 times counterclockwise to mix. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 , if necessary. Incubate at 35 °C for 22-26 hours.
- *Frozen Peas:*
 - **For MP Media Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BAX System MP media. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
 - **For LB Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 , if necessary. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- *Cream cheese:*
 - **For MP Media Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BAX System MP media. Incubate at 35 °C for 12-24 hours.
 - **For LB Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 . Incubate at 35 °C for 22-26 hours.
- *Nonfat dry milk:*
 - **For Brilliant Green Water** - Pour 25 g sample slowly over the surface of 225 mL pre-warmed (35 °C) Brilliant Green 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.
- *Ice cream:*
 - **For LB Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 , if necessary. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.



- **For BPW Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) BPW. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- **For BGW Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35 °C) Brilliant Green Water. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- **Milk-based infant formula:** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 . Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- **Cocoa:**
 - **For 25 g** - Homogenize 25 g sample with 225 mL reconstituted nonfat dry milk. Let stand at room temperature for 55-65 minutes, then swirl thoroughly to mix. Adjust pH to 6.8 ± 0.2 , if necessary. Add 0.45 mL 1% aqueous brilliant green dye solution and mix well. Incubate at 35 °C for 22-26 hours. Transfer 10 µL enrichment to 500 µL BHI broth before processing. *Optional* - Incubate BHI broth at 37 °C for 3 hours.
- **White pepper:** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) TSB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 , if necessary. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- **Orange Juice:**
 - **For MP Media Enrichment** – Swirl 25 mL sample thoroughly with 225 mL pre-warmed (41 °C) BAX System MP media. Incubate at 39 to 42 °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.
 - **For UPB Enrichment** – Swirl 25 mL sample thoroughly with 225 mL pre-warmed (35 °C) UPB. 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.
- **Dry pet food:**
 - **For LB Enrichment** – Homogenize 375 g sample with approximately one-third to one-half of 3375 mL pre-warmed (35 °C) LB. Add the remainder of the pre-warmed media. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 , if necessary. 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.
 - **For BPW Enrichment** - Homogenize 375 g sample with approximately one-third to one-half of 3375 mL prewarmed (35 °C) BPW. Add the remainder of the prewarmed media. Incubate at 35 °C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL prewarmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- **Environmental sponges (Stainless steel, ceramic tile, plastic):**
 - **For LB Enrichment** - Homogenize sponge with 225 mL pre-warmed (35 °C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2 , if necessary. Incubate at 35 °C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
 - **For BPW Enrichment** (225 mL) - Homogenize sponge with 225 mL pre-warmed (35 °C) BPW. Adjust pH to 6.8 ± 0.2 , if necessary. Incubate at 35 °C for 18-24 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37 °C) BHI. Incubate at 37 °C for 3 hours.
- **Dried cannabis and hemp flower:** Combine 10 g flower with 90 mL prewarmed (37 to 42 °C) BPW. Hand massage for 1 minute and incubate at 42 °C for 22-26 hours. Add 10 µL enrichment to 500 µL prewarmed BHI broth (37 °C) and incubate in a 37 °C heat block for 3 hours.



- *Sampling Cloths such as MicroTally® (375 g Beef Trim):*
 - **For MP Media Enrichment:** Combine one sampling cloth with 200 mL pre-warmed (42 °C) MP media. Stomach for 1 minute and incubate at 42 ± 1 °C for 10-24 hours.
 - **For mTSB + caa Enrichment:** Combine one sampling cloth with 200 mL pre-warmed (42 °C) mTSB + caa (modified TSB with casamino acids) media. Stomach for 1 minute and incubate at 42 ± 1 °C for 8-24 hours.

ENRICHMENT PROTOCOL – ACTERO™ ENRICHMENT MEDIA

1. Prepare Enrichment Broth

Prepare Actero™ Elite *Salmonella* Enrichment Media according to the manufacturer's instructions.

2. Collect and Enrich Samples

Method Approved by AOAC

- *Milk Chocolate:* Homogenize 25 g sample with 175 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 22-26 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Chocolate liquor:* Homogenize 25 g sample with 225 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 26-30 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Dry pet food:*
 - **For 25 g:** Homogenize 25 g sample with 225 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 18-22 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
 - **For 375 g:** Homogenize 375 g sample with approximately one-third to one-half of 2625 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Add the remainder of the pre-warmed media. Incubate at 35 °C for 18-22 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Cocoa:* Homogenize 25 g sample with 175 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media supplemented with 5% NFDM. Incubate at 35 °C for 16-20 hours. Transfer 40 µL enrichment to 2 mL PBS and mix before processing.
- *Shell Eggs:* Homogenize by hand a 20 egg sample with 1000 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 16-20 hours. Transfer 40 µL enrichment to 2 mL PBS and mix before processing.
- *Ground Chicken:* Homogenize 25 g sample with 225 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media supplemented with 50 mg/L of malachite green. Incubate at 35 °C for 14-18 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Ground beef:*
 - **For 25 g:** Homogenize 25 g sample with 75 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media supplemented with 50 mg/L of malachite green. Incubate at 35 °C for 16-20 hours.
 - **For 375 g:** Homogenize 375 g sample with 1125 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media supplemented with 25 mg/L of malachite green. Incubate at 39 to 42 °C for 20-24 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Chicken Carcass Rinse:* Homogenize by hand 30 mL of BPW rinse sample with 30 mL pre-warmed (35 °C) Actero *Salmonella* Enrichment Media supplemented with 20 mg/L of malachite green. Incubate at 35 °C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.



- *Dried Whole Egg*: Homogenize by hand 100 g sample with 600 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media supplemented with 5% NFDM. Incubate at 35 °C for 14-18 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Whole Liquid Egg (Pasteurized)*: Homogenize by hand 100 g sample with 300 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Adjust pH to 7.0 ± 0.2 if necessary. Incubate at 35 °C for 18-22 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Raw Almond*: Homogenize 375 g sample with 750 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Dried Raisins*: Homogenize 25 g sample with 75 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Peanut Butter*: Homogenize 25 g sample with 175 mL pre-warmed (35 °C) Actero Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Whole Black Pepper*: Homogenize 25 g sample with 75 mL pre-warmed (35 °C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Dried Parsley*: Homogenize 25 g sample with 225 mL pre-warmed (35 °C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35 °C for 20-24 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- Environmental sponges (Stainless steel and plastic): Homogenize sponge with 90 mL pre-warmed (35 °C) Actero™ *Salmonella* Enrichment Media. Incubate at 35 °C for 14-18 hours. Transfer 40 µL enrichment to 2 mL PBS and mix before processing.

ENRICHMENT PROTOCOL – BAX® SYSTEM SALQUANT®

1. Prepare Enrichment Broth

- BAX System MP Media—Prepare MP media according to manufacturer's instructions.
- BAX System MP Media plus Quant Solution –Prepare MP media according to manufacturer's instructions. Autoclave at 121 °C for 15 minutes, then add 1 mL Quant Solution per liter.

2. Collect and enrich samples

- *Raw comminuted Turkey and Chicken*: Add 325 g portions to 1625 mL BPW. Homogenize by hand until all clumps have been dispersed. Transfer 30 mL of homogenate to a sterile filtered bag. Add 30 mL of prewarmed (42 °C) BAX MP media with novobiocin (40 mg/L) to each sample bag and hand mix for 30 seconds. Incubate samples at 42 ± 1 °C for 8 h. After transferring to BAX MP with novobiocin for BAX System SalQuant enrichment, incubate the remaining original homogenate sample at 35 °C for 24 hours for prevalence testing.
- *Whole Bird Rinsates*: Combine 30 mL poultry rinsate to 30 mL prewarmed (45 °C) BAX MP media plus 1 mL/L Quant Solution. Hand mix for 30 seconds and incubate sample at 42 °C for 6 hours. After pulling aliquot at the determined time point for lysate creation, incubate the remaining original homogenate sample at 42 °C for 18-24 hours for prevalence testing.
- *Raw Ground Beef and Raw Ground Pork*: Add 375 g portions to 1.5 L prewarmed (45 °C) BAX MP media and hand massage until all clumps have been dispersed. Transfer 30 mL of homogenate to a sterile filtered bag. Add 30 mL of prewarmed (45 °C) BAX MP media plus 1 mL/L Quant Solution. Hand mix for 30 seconds and incubate sample at 42 °C for 6 hours for raw ground beef and at 7 hours for raw ground



pork. Incubate the remaining original homogenate sample (375 g in 1,500 mL BAX MP) at 42 °C for 18-24 hours for prevalence testing.

- *Raw Beef Trim and Raw Pork Trim:* Add 375 g portions to 1.5 L prewarmed (45 °C) BAX MP media and hand massage for 30 seconds. Incubate sample at 42 °C for 6 hours. After pulling aliquot at the determined time point for lysate creation, incubate the remaining original homogenate sample at 42 °C for 18-24 hours for prevalence testing.
- *MicroTally on Raw Beef Trim and MicroTally on Raw Pork Trim:* Add one MicroTally cloth to 200 mL prewarmed (45 °C) BAX MP media and hand mix for 30 seconds. Incubate sample at 42 °C for 6 hours. After pulling aliquot at the determined time point for lysate creation, incubate the remaining original homogenate sample at 42 °C for 18-24 hours for prevalence testing

ENRICHMENT PROTOCOL FOR MPN ESTIMATION

- *Raw comminuted Turkey and Chicken:* Homogenize 65 g samples with 585 mL BPW. Make a 3-tube, 5-dilution MPN set representing 1 g, 0.1 g, 0.01 g, 0.001 g, and 0.0001 g of sample by setting up the following: For 1 g sample dilution, fill 3 test tubes with 10 mL homogenate. For 0.1 g sample dilution, fill 3 test tubes of 9 mL BPW with 1 mL sample homogenate. For 0.01 g sample dilution, fill 3 test tubes of 9.9 mL of BPW with 0.1 mL of sample homogenate. For 0.001 g sample dilution, add 0.1 mL of sample homogenate to 9.9 mL BPW, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. For 0.0001 g sample dilution, add 0.1 mL of homogenate to 99.9 mL BPW, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. Incubate tubes at 37 °C for 24 hours. Continue with the creation of lysates for each incubated tube for prevalence testing.
- *Whole Bird Rinsates:* Make 3-tube, 5-dilution MPN set representing 1 g, 0.1 g, 0.01 g, 0.001 g, and 0.0001 g of sample by setting up the following: For 1 mL sample dilution, fill 3 test tubes with 1 mL rinsate and 9 mL BPW. For 0.1 mL sample dilution, fill 3 test tubes of 9 mL BPW with 1 mL from previous sample dilution. For 0.01 mL sample dilution, fill 3 test tubes of 9 mL of BPW with 1 mL from previous sample dilution. For 0.001 mL sample dilution, add 1 mL from previous sample dilution to 9 mL BPW. For 0.0001 mL sample dilution, add 1 mL from previous sample dilution to 9 mL BPW. Incubate tubes at 37 °C for 24 hours. Continue with creation of lysates for each incubated tube for prevalence testing.
- *Raw Beef Trim:* Homogenize 65 g samples with 585 mL mTSB and hand mix for 30 seconds. Make 3-tube, 5-dilution MPN set representing 1 g, 0.1 g, 0.01 g, 0.001 g, and 0.0001 g of sample by setting up the following: For 1 g sample dilution, fill 3 test tubes with 10 mL homogenate. For 0.1 g sample dilution, fill 3 test tubes of 9 mL BPW with 1 mL sample homogenate. For 0.01 g sample dilution, fill 3 test tubes of 9.9 mL of BPW with 0.1 mL of sample homogenate. For 0.001 g sample dilution, add 0.1 mL of sample homogenate to 9.9 mL BPW, vortex, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. For 0.0001 g sample dilution, add 0.1 mL of homogenate to 99.9 mL BPW, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. Incubate tubes at 42 °C for 24 hours. Continue with the creation of lysates for each incubated tube for prevalence testing.

Method Approved by AFNOR Certification

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

- *General Protocol for meat products (including meat with spices or herbs), seafood, and vegetables:* Homogenize 25 g sample with 225 mL pre-warmed (37 °C) BPW. Incubate at 37 °C for 16-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours.



- *Pet food:*
 - **For 25 g** - Homogenize 25 g sample with 225 mL pre-warmed (36 ± 1 °C) BPW. Incubate at 37 °C for 16-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours.
 - **Up to 375 g (1:10)** - Homogenize 375 g sample with 3375 mL pre-warmed (36 ± 2 °C) BPW. Incubate at 36 ± 2 °C for 18-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours. *Optional:* For Free DNA removal, follow the instructions as outlined for BAX System Free DNA Cleanup Kit (KIT2041) in INS2029.
 - **Up to 375 g (1:6)** - Homogenize 375 g sample with 1875 mL pre-warmed (36 ± 2 °C) BPW. Incubate at 36 ± 2 °C for 18-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours. *Optional:* For Free DNA removal, follow the instructions as outlined for BAX System Free DNA Cleanup Kit (KIT2041) in INS2029.
 - *Egg products:* Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37 °C for 18-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours.
 - *Raw beef (short protocol):* Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 41.5 °C for 10-24 hours.
 - *Raw Meats and raw seafood:* Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37 °C for 16-24 hours.
 - *Environmental samples:*
 - **For Swab** - Homogenize 1 swab with 10 mL pre-warmed (36 ± 2 °C) BPW. Incubate at 36 ± 2 °C for 18-26 hours.
 - **For Sponge** - Homogenize 1 sponge with 100 mL pre-warmed (36 ± 2 °C) BPW. Incubate at 36 ± 2 °C for 18-26 hours.
 - *Optional (swab or sponge):* Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours. *Optional:* For Free DNA removal, follow the instructions as outlined for BAX System Free DNA Cleanup Kit (KIT2041) in INS2029.
 - *Dairy Products:* Homogenize 25 g sample with 225 mL pre-warmed (36 ± 2 °C) BPW with 20mg/L novobiocin. Incubate at 41.5 °C for 20-28 hours. *Optional:* For Free DNA removal, follow instructions as outlined for BAX System Free DNA Cleanup Kit (KIT2041) in INS2029.
 - *Chocolate:*
 - **For 25 g** - Homogenize 25 g sample with 225 mL pre-warmed NFDM (reconstituted non-fat dried milk). Let stand for 55-65 minutes. Add 450 µL of 1% Brilliant Green. Incubate at 37 °C for 22-30 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours.
 - *Milk and Dark Chocolate:*
 - **Up to 375 g (1:5)** - Homogenize 375 g sample with 1500 mL pre-warmed (36 ± 2 °C) NFDM (reconstituted non-fat dried milk). Let stand for 55-65 minutes. Incubate at 36 ± 2 °C for 22-30 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours.
 - **Up to 375 g (1:10)** - Homogenize 375 g sample with 3375 mL pre-warmed (36 ± 2 °C) NFDM (reconstituted non-fat dried milk)*. Let stand for 55-65 minutes. Incubate at 36 ± 2 °C for 22-30 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37 °C for 3-4 hours.
 - *Optional:* For Free DNA removal, follow the instructions as outlined for BAX System Free DNA Cleanup Kit (KIT2041) in INS2029.
- *BPW may be used for products with low cocoa content.



Note: Follow General Protocol, for matrices not falling into protocols listed above.

Note: Follow guidelines as appropriate from ISO 6887.

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 to incubation temperature 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 to 37 °C and 95 °C*.
- 3.2 Make sure cooling blocks are chilled to 2 to 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 a 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 the corresponding cluster tube.
- 4.5 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).

- 4.6 Heat at 37 °C for 20 minutes.
- 4.7 Heat at 95 °C for 10 minutes.
- 4.8 Cool at 2 to 8 °C in a chilled cooling block for at least 5 min.

Note: Minimize the time between removing the cooling block inserts from the refrigerator and using them to keep them as chilled as possible. Remember that you must finish using the cooling blocks within 30 minutes of removing them from the refrigerator.

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 to 8 °C) PCR cooling block.

Note: Remember that you must finish using the cooling blocks within 30 minutes of removing them from the freezer.

- 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 30 μ L 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.

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

5.7 After completing the hydration of all PCR tablets, 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

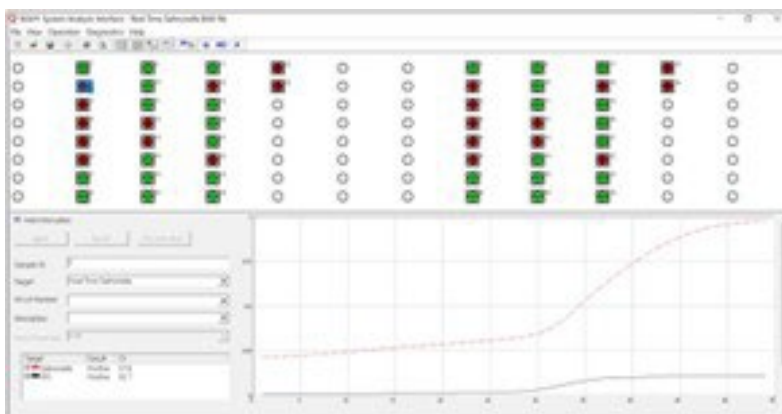
6.1 At the “Ready for Rack Load” prompt, click the NEXT button and open the instrument drawer.

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 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.

FOR BAX System SalQuant ONLY

For quantitative results, upload .bax files into SureTrend®. The .bax files must be generated using BAX Q7 software v5.1 or later. Please contact Hygiena Technical Support if assistance is needed.

FOR BAX MPN method

Utilize MPN tables in USDA-FSIS MLG 2.05 Most Probable Number Procedure and Tables to estimate MPN/g.



CONFIRMATION

Method Approved by AOAC

If desired, BAX System results can be confirmed with the reference culture method appropriate for the sample type, such as:

- US FDA Bacteriological Analytical Manual (BAM)
- USDA FSIS Microbiology Laboratory Guidebook (MLG)
- International Organization for Standardization (ISO)
- AOAC SMPR 2020.002

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

In the context of NF validation technical rules, all samples identified as positive by the BAX Real-Time *Salmonella* method must be confirmed starting from a colony in one of the following ways:

- Any relevant ISO 16140-6 AFNOR-validated confirmation method.
- In the context of NF validation technical rules, all samples identified as positive by the BAX RT *Salmonella* method must be confirmed starting from a colony.
- The reference culture method according to ISO 6579-1 standard, Oxoid *Salmonella* latex test and foodproof *Salmonella* detection LyoKit can be utilized.
- *For General protocol (including meat with spices/herbs) and Egg products:* Streak 10 µL of last enrichment to Brilliance *Salmonella* Agar and XLD and incubate at 37 °C for 22-26 hours. To subculture, transfer 100 µL of last enrichment to 10 mL RVS broth and incubate 41.5 °C for 21-27 hours. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and XLD Agar and incubate at 37 ± 1 °C for 22-26 hours. Confirm presumptive positive colonies with a latex test (Oxoid).
- *For Raw Beef (specific short protocol), Raw Meats and Raw Seafood:* Streak 10 µL of BPW onto Brilliance *Salmonella* Agar and XLD and incubate 22-26 hours at 37 ± 1 °C. To subculture, transfer 100 µL BPW enrichment to 10 mL of RVS broth and incubate 21-27 hours at 41.5 °C. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and XLD and incubate at 37 ± 1 °C for 22-26 hours. Confirm presumptive positive colonies with a latex test (Oxoid).
- *For Dairy Products (except Powdered Milk):* Streak 10 µL of BPW with novobiocin onto Brilliance *Salmonella* Agar and XLD and incubate 22-26 hours at 37 ± 1 °C. To subculture, transfer 100 µL BPW with novobiocin enrichment to 10 mL of RVS broth and incubate 21-27 hours at 41.5 °C. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and XLD and incubate at 37 ± 1 °C for 22-26 hours. Confirm presumptive positive colonies with a latex test (Oxoid).
- *For Chocolates:* Streak 10 µL of last enrichment to Brilliance *Salmonella* Agar and XLD and incubate at 37 °C for 22-26 hours. To subculture, transfer 100 µL NFDM or BHI enrichment to 10 mL RVS broth and incubate 41.5 °C for 21-27 hours. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and incubate at 37 ± 1 °C for 22-26 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 (Oxoid).



Note: According to the ISO 7218 standard, pure cultures should be used for biochemical and serological confirmation, although it is possible to conduct these on single colonies from selective agar plates in some cases (e.g., ISO 6579-1). For molecular amplification formats, the use of mixed cultures may be acceptable if sufficient target organism DNA is present.

Some strains of *Salmonella* belonging to the serovar Dublin, may show weak magenta pigmentation, because of their low esterase activity.

BPW and NFDMM 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.

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 Real-Time PCR Assay for *Salmonella* has been certified by the AOAC Research Institute as Performance Tested MethodSM #081201. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies for foods, hemp and cannabis flowers, and surfaces demonstrated BAX System sensitivity and specificity equal to or better than the reference culture-based methods. Validation studies using the SalQuant method on raw comminuted turkey and chicken, whole bird rinses, raw ground beef and pork, raw beef and pork trim and Microtally on raw beef and pork trim, were comparable to USDA/FSIS MLG 2.05.

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 environmental sponges on stainless steel, ceramic tile and plastic surfaces.

The BAX System Real-Time PCR Assay for Salmonella spp. has been certified NF VALIDATION by AFNOR Certification as #QUA 18/08-03/15 for the detection of Salmonella spp. Validation studies conducted according to ISO 16140-2 standards by comparison to ISO 6579-1/A1 found this test kit's performance to satisfy the ISO 16140-2 standard and NF VALIDATION Technical rules criteria for all foods, pet food and environmental samples (excluding primary production samples).

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> or upon request to a Hygiena representative.

The software version and the BAX System Free DNA Cleanup kit approved in the scope of NF-Validation certification are disclosed in the certificate.

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. You can also call 800-863-6842 in the US, 1- 302-695-5300 outside the US or contact us directly at <https://www.hygiena.com/support>.



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