BAC*Gene Salmonella* spp.

TEST KIT FOR QUALITATIVE REAL-TIME PCR DETECTION OF *SALMONELLA* SPP.

5123221801, 5123221810, 5123221811	BAC*Gene Salmonella* spp. BAC*Gene Salmonella* spp. (HTP) BAC*Gene Salmonella* spp. (10x)	for use on Agilent AriaMx™, Bio Rad CFX96 Touch™ and CFX96 Touch™ Deep Well PCR platforms

For 96, 10x 96 (high throughput (HTP) kit) or 10x 96 reactions

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PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 121501

NF VALIDATION EN ISO 16140

EGS 38/01-03/15
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
www.afnor-validation.com

GOLD STANDARD DIAGNOSTICS

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1 INTRODUCTION

Salmonellosis is the second most common foodborne disease within the European Union. Between 90 000 to 100 000 thousand cases per year are reported across member states. Of those, around 900 to 1 500 result in outbreaks. Besides the severe effects on the individual and population health, the presence of *Salmonella* in food and feed also boasts dire effects on the EU economy with costs believed to reach around $300M yearly as consequence of this pathogen. Many foods are prone to contain *Salmonella* species, with eggs and egg products, bakery products and pig meat being some of the most likely to cause outbreaks.

Though there are upwards of 2 000 serovars of *Salmonella*, current law and regulations consider each one as a potential human pathogen and therefore methods of detection must be able to detect all of them. Additionally, there is zero tolerance for its presence in food as though there are some efforts towards quantify *Salmonella* in poultry meat, the majority of regulation require total absence of *Salmonella* species.

Classical methods of detection rely on cultivating these species on agar plates. Although they are still widely used, these methods demand several days to reach a result. Notwithstanding, most industries require considerable faster results to be able to release their products faster or to initiate any required corrective actions as soon as possible in case of the presence of a pathogen.

The advent of DNA-based methods, decreased the time to result exponentially. BAC*Gene Salmonella* spp., relies on the amplification of *Salmonella* specific DNA using real-time polymerase chain reaction (PCR) and its real-time detection via fluorescent probes to obtain results of presence or absence of the pathogen in a couple of hours after the enrichment of a given sample.

1.1 Intended Use

The BAC*Gene Salmonella* spp. detection kit provides materials for the qualitative detection of *Salmonella* spp. DNA from food, feed (including pet food) and environmental samples when monitoring production processes.

It is intended that this kit is used by trained laboratory personnel.

The BAC*Gene Salmonella* spp. kit is validated for use with the Agilent AriaMx™, Bio Rad CFX96 Touch™ and CFX96 Touch™ Deep Well PCR platforms.

The BAC*Gene Salmonella spp*. kit can be run on the OT-2 liquid handler platform from Opentrons Labwork Inc., supporting the automated liquid handling from sample transfer, lysis up to PCR plate preparation.

1.2 Certifications

1.2.1 AFNOR Certification

The BAC*Gene Salmonella* spp. kit is certified by AFNOR Certification in a manual workflow as an alternative method for *Salmonella* spp. detection all human food products, feed and environmental samples according to EN ISO 16140:

- 25 g samples of all human food products including:
  - Meat and meat products
  - Milk and dairy products
  - Produces (vegetables, fruits and related products)
  - Eggs and egg products
  - Fish and seafood products
- 25 g of feed (including pet food)
- up to 375 g of pet food
- up to 375 g of milk powders & infant formula (with and without probiotics)
- up to 375 g heat-processed milk and dairy products (with PR*Eraser**)
- Environmental samples (with PR*Eraser**)

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*PR*Eraser* BAC*Gene* (see sections 1.5 and 2.5.1) has been performance tested for three relevant matrices (heat-process milk and dairy products and production environmental samples).

The end of the validity of the NF VALIDATION certification is indicated on the certificate EGS 38/01-03/15.

1.2.2 AOAC Performance Testing

The BAC*Gene Salmonella* spp. kit is certified by AOAC-Research Institute in a manual workflow under the Performance Tested Methods[SM] Program for detection of *Salmonella* spp. in:

- Fresh raw ground beef (15% fat, 25 g)
- Frozen spinach (25 g)
- Pasteurized whole liquid eggs (25 mL)
- Frozen cod fillet (25 g)
- Raw whole milk (25 mL)
- Ice cream with chocolate inclusions (25 g, with PR*Eraser**)
- Dog pâté (composed of beef meat and animal by-products, cereals, carrots and vegetable by products, up to 375 g)
- Dry dog pellets (25 g)
- Pet food dry pellets (375 g, with PR*Eraser**)
- Infant formula milk powder supplemented with probiotics (*Bifidus lactis*) (up to 375 g)
- Process water from scalding tank (25 mL)
- Stainless steel environmental surface (1" x 1" area)
- Ceramic tiles environmental samples (4" x 4" area with sponge with PR*Eraser**)
- Cocoa powder, cocoa liquor, cocoa butter, cocoa crumb and milk chocolate (up to 375 g each)
- Ground beef (up to 375 g)
- Ground pork (up to 250 g)
- Marinated chicken with brine (up to 250 g)
- Pork carcass cloth

The end of the validity of the AOAC-RI certification is indicated on the certificate no. 121501.

*PR*Eraser* BAC*Gene* (see sections 1.5 and 2.5.1) has been performance tested for three relevant matrices. Therefore, provided that additional matrices are verified, PR*Eraser* BAC*Gene* can be applied to other matrices within the scope of the method.

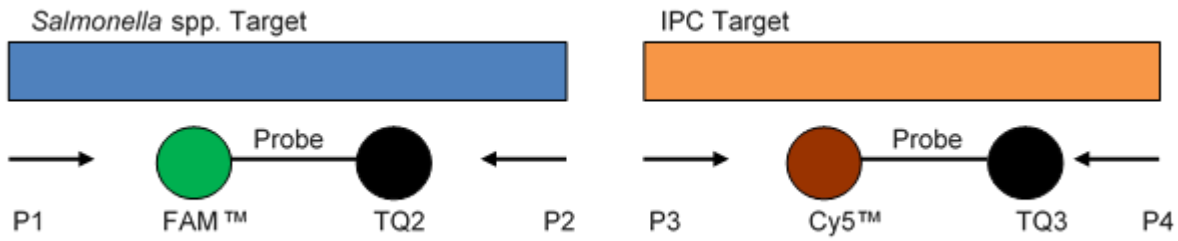
1.3 Test Principle

DNA amplification and detection methods take advantage of the nucleotide sequence conservation found in bacterial genomes that ensures the potential for high specificity and sensitivity in detection of food-borne, pathogenic bacteria.

After enrichment, the microbial DNA is extracted by a simple thermal lysis step and rapidly analysed by real-time PCR. In this way *Salmonella* species are detected from enrichment cultures with extraordinary high sensitivity.

By means of specific primers (P) a nucleotide sequence of the species *Salmonella* is amplified during PCR. Primers do not react with DNA derived from closely related species from the *Enterobacteriaceae* family.

The amplified fragments are detected with a FAM™ fluorescence-labelled hybridization probe quenched by non-fluorescent Tide Quencher™ 2 (TQ2). An internal positive control (IPC) is included in the MasterMix. IPC DNA is amplified in parallel and detected using a Cy5™ fluorescence-labelled hybridisation probe quenched by non-fluorescent Tide Quencher™ 3 (TQ3). IPC detection indicates the proper functioning of the PCR.



1.4 Components of the Kit

Important Notes:

- Store all reagents as indicated below, through to the expiration date printed on the label
  Never store components of the kit together with samples or post-PCR products. Shelf life is indicated on the label of the components.
- Do not mix components from different kit lots.

Cat. no. 5123221801 (96 reactions)

For Lysis:

- 1x **Lysis plate** for sample preparation, empty, rippable (high profile)
- 1x **Domed caps***, for use with lysis plate, set of 12 strips
- 2x **Lysis Buffer I**, 4 mL, –20 °C ± 2 °C or 4 °C ± 2 °C; after addition of proteinase K store at 4 °C ± 2 °C for maximum 2 weeks
- 2x **Proteinase K**, vials with blue cap, each with 500 µL, store at –20 °C ± 2 °C

For PCR:

- 1x **BAC*Gene Salmonella* spp PCR plate**, with pre-dispensed MasterMix and PCR support grid. Store light protected at –20 °C ± 2 °C
- 1x **Optical caps**, for use with PCR plate (1 bag with 120 strips)
- 2x ***Salmonella* positive control plasmid DNA**, vial with yellow cap, 50 µL, store at –20 °C ± 2 °C
  Note: Do not freeze/thaw more than 6 times.

Cat. no. 5123221811 (10x HTP)

For Lysis:

- 10x **Lysis plate** for sample preparation, empty, rippable (high profile)
- 10x **Domed caps***, for use with lysis plate, set of 12 strips
- 2x **Lysis Buffer I**, 40 mL, –20 °C ± 2 °C or 4 °C ± 2 °C; after addition of proteinase K store at 4 °C ± 2 °C for maximum 2 weeks
- 2x **Proteinase K**, 5 mL, store at –20 °C ± 2 °C

For PCR:

- 10x **BAC*Gene Salmonella* spp PCR plate**, with pre-dispensed MasterMix and PCR support grid. Store light protected at –20 °C ± 2 °C
- 10x **Optical caps**, for use with PCR plate (1 bag with 120 strips)
- 4x ***Salmonella* positive control plasmid DNA**, vial with yellow cap, 50 µL, store at –20 °C ± 2 °C
  Note: Do not freeze/thaw more than 6 times.

Cat. no. 5123221810 (10x 96 reactions)

For Lysis:

- 10x **Lysis plate** for sample preparation, empty, rippable (high profile)
- 10x **Domed caps***, for use with lysis plate, set of 12 strips
- 20x **Lysis Buffer I**, 4 mL, –20 °C ± 2 °C or 4 °C ± 2 °C; after addition of proteinase K store at 4 °C ± 2 °C for maximum 2 weeks
- 20x **Proteinase K**, vials with blue cap, each with 500 µL, store at -20 °C ± 2 °C

For PCR:

- 10x **BAC*Gene Salmonella* spp PCR plate**, with pre-dispensed MasterMix and PCR support grid. Store light protected at –20 °C ± 2 °C
- 10x **Optical caps**, for use with PCR plate (1 bag with 120 strips)
- 20x ***Salmonella* positive control plasmid DNA**, vial with yellow cap, 50 µL, store at –20 °C ± 2 °C
  Note: Do not freeze/thaw more than 6 times.

*__Note__: additional domed caps for lysis can be purchased in a package of 250x 8 cap strips (cat. no. 5613900301).

1.5 Additional Equipment, Consumables and Reagents Required

Equipment

For Sampling/Enrichment:

- **Swabs/sponges/wipes/cloths** (for environmental samples)*
  - Gold Standard Diagnostics EZ Reach™ Sponge Sampler, Polyurethane, 10 mL HiCap Neutralizing Broth, 24 oz. bag, 100 pcs (EUR EZ-10HC-PUR)
- **Stomacher® or paddle blender**, capable of running between 15 000 and 20 000 rev/min
- **Enrichment bags** with side filter

For Lysis:

- 2x **Block heater** (e.g. block heater, digital, VWR)
- 2x **Insert for block heater**, 0.2 mL, 96 wells (e.g. double block, 1×96-well PCR plate, VWR)
- 1x **Lysis support grid**, blue, (e.g. cat. no. 5617702209, pack of 8 grids)
- 1x **96 well cooling block for lysis**, blue (e.g. cat. no. HU0060302)
- 1x **Lysis rack, blue – lid included** (e.g. cat. no. 5613901401)
- 1x **Strip centrifuge**:
  - Capacity of 2x 8-well strips: (e.g. Carl Roth GmbH, Rotilabo® centrifuge with butterfly rotor)
  - Capacity of 4x 8-well strips: (e.g. IKA, Mini Centrifuge Mini G or VWR, MiniStar silverline)

  OR
- 1x **Plate centrifuge**:
  - Capacity of two times 12x 8-well strips: (e.g. Benchmark Scientific, PlateFuge™ microplate microcentrifuge)
- 1x **Capping/Uncapping tool** for 8-well strips (e.g. MicroAmp® Cap Installing Tool, cat. no. HU0060301)
- 1x **Stepper pipette** (with 1 mL tip), (e.g. HandyStep® S Brand®)
- 1x **Single channel pipette**, (e.g. Brand®, Transferpette® S 100 – 1 000 µL)
- 1x **Single channel pipette**, (e.g. Brand®, Transferpette® S 10 – 100 µL)
- 1x **Single channel pipette**, (e.g. Brand®, Transferpette® S, 0.5 – 10 µL)
- 1x **Pipette filler**, (e.g. VWR, Pipetboy acu 2 for serological pipettes)

For PCR:

- 1x **PCR support grid**, yellow (e.g. cat. no. 5617702208, pack of 8 grids)
  Note: 1x PCR support grid is provided with each kit.
- 1x **96 well cooling block for PCR**, pink (e.g. cat. no. HU0060303)
- 1x **PCR rack, yellow – lid included** (e.g. cat. no. 5613901301)
- 1x **Capping/Uncapping tool** for 8-well strips (e.g. MicroAmp® Cap Installing Tool, cat. no. HU0060301)
- 1x **Single channel pipette**, (e.g. Brand®, Transferpette® S, 0.5 – 10 µL)

  AND/OR
- 1x **Multichannel pipette**, (e.g. Brand®, Transferpette® S-8 Kanal/0.5 – 10 µL)
- 1x **Disposable transfer rack** (e.g. an empty tip box) as mount for the yellow support grid and the PCR plate
- 1x **Real-time PCR Thermocycler**:
  - Agilent AriaMx™ (up to Software v.2.0), with FAM™ and Cy5™ filter set
  - Bio-Rad CFX96 Touch™ (CFX Manager™ Software v. 3.1/CFX Maestro™ Software 2.3)
  - Bio-Rad CFX96 Touch™ Deep Well (CFX Manager™ Software v. 3.1/ up to CFX Maestro™ Software 2.3)
- **Windows 7/10 PC with Microsoft Excel** 2010 32 bit (Excel Version 14.7140.xxxx or later)
- **BAC*Gene* Evaluation Sheet Version 2**

  OR

  FastFinder automated PCR analysis software (UgenTec NV, Hasselt, Belgium) with BAC*Gene Salmonella* spp. plugins with version 1

  OR

  PURE Software Version 1 (Gold Standard Diagnostics)

Reagents

For Sampling/Enrichment:

- **Neutralizing buffer**, e.g.
  - Dey-Engley
  - Letheen broth
  - HiCap broth
- **MRB medium** (for carcass sampling)
- **Diluents** according to EN ISO 6887-1 (for carcass sampling)
- **Buffered Peptone Water (BPW)**, prepared according to EN ISO 6579 and EN ISO 11133 standards
- **UHT milk** (for cocoa products primary enrichment)
- **Brilliant Green** (for cocoa products primary enrichment)
- **BHI medium** (for cocoa products secondary enrichment)
- **Tween-80®** (polysorbat 80; polyoxyethylene (20) sorbitan monooleate, CAS-Number 9005-65-6) for products containing >20% fat, unless the products already contain sufficient emulsifier.
  - Add sufficient amount of Tween-80® to improve emulsification during suspension (diluent between 1 g/L and 10 g/L, according to estimated fat content).
  - Alternative surfactants and emulsifiers are available under various trade names, but the proportions to use should be determined by the laboratory (according to EN ISO 6887- 4 :2017).

Consumables and material not contained in the kit

For Sampling/Enrichment:

- **PR*Eraser* BAC*Gene***, cat. no. 5123222301 (1x 96) or 5123222311 (10x 96)
  - For the sample pre-treatment after enrichment and prior to lysis

Note: For information on certified matrix scope, please refer to section 1.2.1 for NF validation certification and 1.2.2 for AOAC-PTM certification.

For Lysis:

- **Pipette tips**, DNA-/Nuclease-free pipette tips
- **Stepper tips,** 1 mL
- **Sterile container** for enrichment storage
- **Serological pipettes**
- **Gloves**, powder-free

For PCR:

- **Pipette tips**, DNA-/Nuclease-free pipette tips
- **Nuclease free water**, molecular biology grade
- **Gloves**, powder-free

2 HOW TO USE THIS PRODUCT

2.1 Safety Precautions

- All samples should be handled with caution as they are potentially infectious.
- In order to safeguard the health of laboratory personnel, it is essential that the whole of this method be carried out only by skilled personnel using good laboratory practices.
- A BSL-2 laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens.
- Relevant national Health and Safety Regulations relating to this organism must be adhered to.
- Care must be taken in the disposal of all infectious materials.
- Do not eat, drink or apply cosmetics in the work area in which the test is performed.
- Do not pipette by mouth.
- Avoid contact of kit components with injured skin.
- *Salmonella* spp. should not be handled by pregnant women, children, the elderly and immuno-compromised individuals due to the high infection risk and fatal health consequences for this group, in particular for the unborn child in case of pregnant women.
- The BAC*Gene Salmonella* spp. kit contains proteinase K which may cause allergic reactions.
- Lysis Buffer I can cause serious eye irritation.
- Always wear appropriate Personal Protective Equipment (PPE). The remaining components of the BAC*Gene Salmonella* spp. kit are not classified according to Regulation (EC) No. 1907/2006 (REACH). For more information, please refer to the kit's safety data sheet (SDS).

2.2 Working Guidelines

- Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).
- Refer to EN ISO 22174:2005 for the general requirements for the in vitro amplification of nucleic acid sequences.
- Refer to associated guidelines for lab equipment maintenance e.g. ISO/DIS20836 for thermal performance testing of thermal cyclers.
- Perform cleaning protocol (outlined in next section).
- Clearly separate the area for taking samples from the enrichment, from the area where lysis and particularly PCR setup are performed.
- Use different equipment (e.g. pipettes) for sampling the enrichment, performing lysis and PCR
- Use DNA, nuclease-free and sterile lab ware.
- Wear gloves.
- Change gloves after removing samples from the enrichment, before starting the lysis protocol.

2.2.1 Cleaning Protocol

Assuring workspaces are clean, before, during and after working with BAC*Gene* is key to ensure the validity of the results obtained.

Efficiently cleaning needs to ensure the disinfection and decontamination of the working area, to avoid:

- Bacterial contamination – prevented by regular disinfection,
- DNA contamination – prevented by regular decontamination.

Please refer to the ISO 7218:2007 for regular disinfection procedures, to contribute towards the safety of the laboratory personnel by preventing risks of infection and to ensure the validity of food microbiology examinations by ensuring that the general techniques used for conducting these examinations are standardised, helping achieve homogeneous results in different laboratories.

For DNA decontamination of surfaces, see below examples of suitable cleaning agents and respective suppliers. We do not take any guarantees if the end user does not strictly follow the instructions for use or does not use recommended procedures by the cleaning agents' suppliers.

Cleaning Agent	**Supplier**
ROTI®Nukleinsäurefrei	Carl Roth GmbH
DNA Away	ThermoFisher Scientific™
DNA-ExitusPlus™	VWR

2.3 Waste Disposal

Dispose of any waste which is potentially contaminated with pathogenic bacteria according to your internal and local regulations.

2.4 Enrichment

For preparation of test samples and initial suspensions follow instructions of EN ISO 6579, EN ISO 6887, EN ISO 17604, EN ISO 7218 and EN ISO 18593 standards. Especially for higher sample volumes it may be applicable to homogenise samples by hand massage, otherwise use a stomacher.

It is strongly recommended to prepare samples in stomacher bags containing a side filter. It is also strongly recommended to prepare a low or non-toxigenic and easily identifiable positive enrichment control (e.g. *Salmonella* Tranoroa derived from ATCC® 700148 or if allowed by local law the UV biotagged strain *Salmonella* Tranoroa derived from FDA Sal91, Microbiologics, cat. no. 01281BUV) and negative enrichment control in parallel with the samples. *Salmonella* Tranoroa can be identified from the enrichment broth using BAC*Gene Salmonella* Tranoroa detection kit (cat. no. 5123222801). Growth in the negative enrichment control indicates contamination of the medium used and therefore enrichment should be repeated.

Note: For sampling of the enrichment it is highly recommended to use filter foams which can be applied to the serological pipets.

After the enrichment protocol, samples can be stored at 2 – 8 °C for up to 72 h before testing.

Certified matrices by AOAC (License No: 121501)					
Matrix	**Enrichment**			**Volume for Analysis**	
	Medium	**Temp**	**Time**	**Enrichment**	**Lysate**
Fresh raw ground beef (15% fat) Frozen Spinach Pasteurized whole liquid eggs Frozen cod fillet **25 g**	1:10 BPW	37 ± 1 °C	16 – 24 h	10 µL	5 µL
Dry dog pellets **25 g**			18 – 24 h		
Process water from scalding tank **25 mL**					
Ice cream with chocolate inclusions **25 g** (incl. PR*Eraser*)					
Pet food dry pellets **375 g** (incl. PR*Eraser*)					
Stainless steel environmental surface **1" x 1" area**	10 mL BPW				
Ceramic tiles environmental samples **4" x 4" area with sponge** (incl. PR*Eraser*)	100 mL BPW				
Infant formula milk powder supplemented with probiotics (*Bifidus lactis*) **up to 375 g**	1:10 double strength BPW (pre-warmed to 37 ± 1 °C)				
Dog pâté (composed of beef meat and animal by-products, cereals, carrots and vegetable by products **up to 375 g**	1:10 BPW (pre-warmed to 37 ± 1 °C)				
Raw whole milk **25 mL**	1:10 BPW	41.5 ± 1 °C			

Certified matrices by AOAC (License No: 121501): Rapid protocols of selected meat matrices & carcass cloths					
Matrix	**Enrichment**			**Volume for Analysis**	
	Medium	**Temp**	**Time**	**Enrichment**	**Lysate**
Ground beef **up to 375 g**	1:10 BPW (pre-warmed to 41.5 ± 1 °C)	41.5 ± 1 °C	10 – 18 h	50 µL	5 µL
Ground pork **up to 250 g**					
Pork carcass cloths Pre-moistened with MRB **1 cloth**	90 mL BPW (pre-warmed to 41.5 ± 1 °C)				
Marinated chicken with brine **up to 250 g**	1:10 BPW (pre-warmed to 37 ± 1 °C)	37 ± 1 °C			
Pork carcass cloths Pre-moistened with diluents (according to 6887-1) **1 cloth**	90 mL BPW (pre-warmed to 37 ± 1 °C)				

Certified matrices by AOAC (License No: 121501): For cocoa-containing products up to 375 g								
Matrix	**Primary Enrichment**			**Secondary Enrichment**			**Volume for Analysis**	
	Medium	**Temp**	**Time**	**Medium**	**Temp**	**Time**	**Enrich-ment**	**Lysate**
Cocoa powder Cocoa liquor Milk chocolate Cocoa butter Cocoa crumb **up to 375 g**	1:10 skimmed/non-fat milk (0.1 – 0.3% fat) (pre-warmed to 37 ± 1 °C) + Brilliant Green (0.018 g/L)	37 ± 1 °C	18 – 24 h	10 µL primary + 500 µL BHI (room temperature*)	41.5 ± 1 °C	3 – 5 h	10 µL (of 2nd enrich-ment)	5 µL

**Room temperature (20-25 °C)*

Note (uncertified protocol):

For nut chocolate pastes (e.g. hazelnut cocoa spreads, praline fillings and similar, excluding chocolates with whole nuts or nut pieces) it is recommended to follow a modification of the cocoa protocol.

It has been demonstrated that replacing Buffered Peptone Water by skimmed / non-fat milk (0.1 – 0.3% fat) results in a significant increase in *Salmonella* growth for these matrix items.

Incubation times (18 – 24 h) and temperature (37 ± 1 °C) as well as the BHI step (at 41.5 ± 1 °C for 3 – 5 h) shall be performed as in the above mentioned cocoa protocol. Protocol is not within AOAC PTM certification.

<table>
<tr><th colspan="6">Certified categories by AFNOR (EGS 38/01-03/15)</th></tr>
<tr><th rowspan="2">Sample Category</th><th colspan="3">Enrichment</th><th colspan="2">Volume for Analysis</th></tr>
<tr><th>Medium</th><th>Temp</th><th>Time</th><th>Enrichment</th><th>Lysate</th></tr>
<tr><td>All human food products
(except Milk & Dairy)
25 g</td><td rowspan="3">1:10 BPW</td><td rowspan="11">37 ± 1 °C</td><td>16 – 24 h</td><td rowspan="12">10 µL</td><td rowspan="12">5 µL</td></tr>
<tr><td>Feed products (incl. pet food)*
25 g</td><td rowspan="11">18 – 24 h</td></tr>
<tr><td>Dusts, process water
25 g/mL</td></tr>
<tr><td>Swab
1
(incl. PREraser)</td><td>10 mL BPW</td></tr>
<tr><td>Wipe, cloth
1</td><td>225 mL BPW</td></tr>
<tr><td>Sponge
1</td><td>100 mL BPW</td></tr>
<tr><td>Milk powders & infant formula without probiotics
up to 375 g</td><td>1:10 BPW (pre-warmed to 37 ± 1 °C)</td></tr>
<tr><td>Milk powders & infant formula containing probiotics
up to 375 g</td><td>1:10 double strength BPW (pre-warmed to 37 ± 1 °C)</td></tr>
<tr><td>Heat-processed milk and dairy products
up to 375 g
(incl. PREraser)</td><td>1:10 double strength BPW (pre-warmed to 37 ± 1 °C)</td></tr>
<tr><td>Pet food
up to 375 g</td><td>1:10 BPW (pre-warmed to 37 ± 1 °C)</td></tr>
<tr><td>Milk & Dairy
25 g/mL</td><td>1:10 BPW</td><td>41.5 ± 1 °C</td></tr>
</table>

***Note:** Storage at 2 – 8 °C for up to 72 h is not certified for this category*

2.5 Before You Begin

1. Place cooling block for lysis at 4 °C ± 2 °C overnight.
2. Turn on heating block to 37 °C ± 2 °C and 95 °C ± 2 °C.
3. Thaw proteinase K just before adding it to Lysis Buffer I
4. Prepare final lysis buffer*:
   For cat no. 5123221801 (1x 96) and cat. no. 5123221811 (10x 96):
   Add 500 µL proteinase K (vial with blue cap) to 4 mL Lysis Buffer I (transparent container).

   For cat no. 5123221810 (HTP):
   Add 5 mL proteinase K (small transparent container) to 40 mL Lysis Buffer I.
5. Mix gently by inverting 5 times.
6. Dispense the final lysis buffer by placing the lysis plate into the lysis rack and aliquoting 90 µL (**Exceptions**: 50 µL for ground beef up to 375 g, ground pork up to 250 g, marinated chicken with brine up to 250g and pork carcass cloths) of the final lysis buffer into each well.
7. Seal wells with domed caps.
8. Mark date of preparation and name on the label provided with kit and place onto the storage rack for lysis buffer plate/strips.
9. The final lysis buffer can be stored for 2 weeks at 4°C ± 2°C.

***Note:** The volume of Lysis Buffer I of the kits with cat nos. 5123221801 and 5123221811 (4.5 mL) is sufficient for 48 reactions. Lysis Buffer I volume of the kit with cat no. 5123221810 (45 mL) is sufficient for 480 reactions or five 96 well plates.

2.5.1 Sample Pre-treatment with PR*Eraser* BAC*Gene*

An optional elimination of free DNA from enrichments prior to the lysis step can be incorporated into the workflow and has been AOAC-RI certified with the use of the kit.

PR*Eraser* BAC*Gene* can be applied for each sample in case of sample types with expected high levels of free DNA (e.g. environmental samples) or after a first PCR positive prior to a second BAC*Gene* PCR to exclude the presence of free DNA.

Please refer to the PR*Eraser* BAC*Gene* kit instruction for use to include PR*Eraser* BAC*Gene* to the workflow.

For information on certified matrix scope, please refer to section 1.2.1 for NF validation certification and 1.2.2 for AOAC-PTM certification.

See section 1.5 for ordering information.

2.6 Sample Lysis

1. Label lysis buffer plate/strips to ensure correct orientation when pipetting samples.
2. Using a serological pipette, remove an aliquot of enrichment culture and dispense into a sterile container. This step is highly recommended to avoid cross-contamination, as opposed to inserting a micropipette directly into the enrichment bag.
3. Use an Un-/Capping Tool (with blue lysis label, dedicated to lysis area) to open the first lysis strip.

   Note: Open each strip individually, add samples and close. Only then proceed to the next strip. The correct method to use this tool is to place the "teeth" of the tool under the connection between the caps and lever the caps open.
4. Transfer 10 – 50* µL of enrichment into appropriate wells.

   ***Exceptions**:

   For up to 375 g meat samples and pork carcass cloths transfer 50 µL of enriched sample to a lysis well containing 50 µL of final lysis buffer.

   Note: Do not add enrichment samples to wells A1 and B1 to ensure the same layout in the following PCR (A1: positive control, B1: negative control).
5. Seal the strip using the Un-/Capping Tool.
6. Proceed to the next lysis strip until all samples have been included.
7. Place lysis plate/strips with blue frame onto the 37 °C (± 2 °C) heating block for 20 min.
8. Transfer lysis plate/strips with blue frame onto the 95 °C (± 5°C) heating block for 10 min.
9. Finally transfer lysis plate/strips with blue frame onto the blue 96 well cooling block (stored at 4 °C ± 2 °C) for 5 min.
10. Ensure that no condensation remains in the lids by transferring the lysis plate/strips with the blue frame to a 96-well plate centrifuge or a centrifuge with adapter for 0.2 mL in 8-strip format. Spin down samples for 30 sec within the range of 400 x g to 2 000 x g. If condensate remains in the lids, please repeat this step.
11. If particles are visible in the lysis vessel, centrifuge the plate/strip following the procedure in the step above.
12. Transfer lysis plate and blue frame into the working rack with transparent inlay.

2.7 PCR

2.7.1 Special Precautions During PCR Analysis

PCR is an exponential reaction. The detection of single DNA targets is possible. The extreme sensitivity requires special precautions for handling and equipment. After a successful amplification, several billion amplicons are present in the reaction tube. Each of them might lead to a false positive result when contaminating sample material, i.e. by spreading in aerosols.

The most important rules to avoid PCR contamination:

- Separate the different procedures spatially.
  Ideally use separate rooms for sample preparation and PCR setup, or at least dedicated different areas, equipment and consumables for each procedure.
- Use DNA/Nuclease-free filter tips.
- Wear disposable powder-free gloves.
- Store BAC*Gene Salmonella* spp. kit components for PCR and lysis only in dedicated areas, and apart from enrichment preparation areas and sample storage.
- Always use PCR controls and preferably also enrichment controls.
- Consider the PCR/post-PCR area/room where the PCR cycler is located as potentially contaminated with DNA-, decontaminate regularly and make sure to avoid carry over to other areas by using separate equipment, lab coats, shoes.
- Dispose of used PCR plates/strips very carefully, make sure that caps do not open, move waste only in tightly closed bags.
- Use separate equipment and materials for cleaning of different areas, in particular for the PCR cycler room/area and for PCR setup. Instruct cleaning personnel accordingly.
- Control all areas for DNA/amplicon contamination on a regular basis (swabs/PCR analysis).

2.7.2 General Information

PCR is performed in a volume of 25 µL in 0.1 mL reaction tubes/plates according to the real-time PCR cycler instructions.

Samples and controls for BAC*Gene Salmonella* spp. assay

Designation	Volume of MasterMix	Addition of
Test samples	20 µL	5 µL lysate per reaction
Positive control (C+)	20 µL	5 µL *Salmonella* positive control plasmid DNA
Negative control (C-)	20 µL	5 µL heat-inactivated lysis buffer* (prepared alongside samples in well B1, 37 °C for 20 min and 95 °C for 10 min)

2.7.3 Control Reactions

Important

For every PCR, it is necessary to prepare a positive and a negative control reaction. These are required for the evaluation of the PCR results! The layout should start with the positive control in A1, the negative control in B1, samples in the following wells.

Required:

- 1 Positive control (C+)
  5 µL *Salmonella* positive control plasmid DNA
- 1 Negative control (C-)
  5 µL heat-inactivated lysis buffer added to MasterMix

Recommended:

- 1 Negative enrichment control (E-):
  Salmonella free enrichment medium control

2.7.4 PCR

Plate Setup*

The following plate document shows the recommended distribution of reactions:

	1	2	3	4	5	6	7	8	9	10	11	12
A	C+	7	15	23	31	39	47	55	63	71	79	87
B	C-	8	16	24	32	40	48	56	64	72	80	88
C	1	9	17	25	33	41	49	57	65	73	81	89
D	2	10	18	26	34	42	50	58	66	74	82	90
E	3	11	19	27	35	43	51	59	67	75	83	91
F	4	12	20	28	36	44	52	60	68	76	84	P-*
G	5	13	21	29	37	45	53	61	69	77	85	E-
H	6	14	22	30	38	46	54	62	70	78	86	E+

Plate layout for 91/92 samples;
C+ = positive control
C- = negative control
E- = negative enrichment control
E+ = positive enrichment control
P- = negative control for PREraser treatment

**The use of a PREraser negative control (C- or E- with PREraser) is recommended when applying a PREraser treatment to the samples.*

PCR Setup

Before you Begin

- Make sure you have switched on the computer, the PCR instrument and ensure the sample layout for the PCR plate is suitably documented and programmed.
- Only remove the number of PCR strips, containing pre-dispensed MasterMix, necessary for testing the amount of samples to be analysed and store the remainder at –20 °C ± 2 °C.
- **Important:** In case of cutting an 8-strip into smaller parts it is crucial to let the plastic warm up at room temperature for around 1 minute prior to cutting. Do not place the strip into a cooling block.
- **Note:** Frequent freezing and thawing might cause inactivation of the reagents. Do not freeze/thaw more than 3 times.
- Spin down the strips with MasterMix for 30 sec between 400 x g to 2 000 × g.
- Visually check that all MasterMix is in the bottom of the wells.

1. Place PCR strips, together with the yellow PCR support grid, into an appropriate support rack. Avoid exposing the PCR plate to light for long periods of time.
2. The lysis and PCR plate should be arranged in the same orientation in order to facilitate handling with the multichannel pipette and in order to avoid cross contamination of wells (place the lysis plate i.e. behind the PCR plate).
3. Open lysis plate as described in section 2.6.
4. Open the corresponding PCR strip by fixing the upper and lower end with one hand and removing the foil starting with the loose end from top to bottom in 180-degree angle (see Figure 1A).

   Note: Open each strip individually, add samples and close. Only then proceed to the next strip.
5. Transfer 5 µL of each lysate by using an 8-well multichannel pipette to the corresponding PCR strip.

   Note: Make sure that the lysate is taken from the upper half of the lysis volume, avoid touching a potential pellet of debris. Ensure the lysate is pipetted directly on top of the MasterMix.
6. Add 5 µL positive control DNA (vial with yellow cap) to well A1.

   Note: Do not freeze/thaw positive control more than 6 times.
7. Close the PCR strip with an optical cap strip with clean, gloved hands (see Figure 1C).
8. Re-close the lysis strip with fresh domed caps (not supplied with the kit).
9. Repeat steps 3 - 8 with the remaining strips.
10. Homogenise mix thoroughly by vortexing for 10-30 sec or manually shaking (5x)

    Note: Ensure plate is properly closed by the optical caps
11. Briefly spin down
12. Transfer the PCR plate to the real-time instrument and start the run (refer to section 3 for Agilent AriaMx™ and section 4 for Bio-Rad CFX96 Touch™).
13. Store the lysis plate at –20 ± 2 °C in case a re-run of the PCR is required.

Figure 1. A. Removing foil from PCR strips. B. Incorrect way to close lids in one direction. C. Correct way to close lids by first softly fixing the ends of the cap strip to the reaction tubes, then applying even pressure to close the PCR strips tightly

3 AGILENT ARIA MX™: INSTRUCTIONS FOR USE WITH BAC*GENE SALMONELLA* SPP. KIT

The BAC*Gene Salmonella* spp. kit is validated for the use on the Agilent AriaMx™ with software versions up to 2.0.

The PCR platform should be prepared prior to commencing the practical work. For a more detailed description of the instrument programming please refer to the user manual of the Agilent AriaMx™.

BAC*Gene* offers three methods for evaluating the final results. Users can choose between a manual data interpretation using the BAC*Gene* Evaluation Sheet or two automated solutions using FastFinder or PURE Software.

3.1 Running a PCR

The following instructions outline how to set up a PCR run on the Agilent AriaMx™ using an external computer.

Note: If you are using the cycler via the touch screen without an external computer please contact your local sales support for more information.

Note: The Aria software is able to interface with a Laboratory Information Management System "LIMS". Please contact your local sales support for more information.

3.1.1 Running PCR Using a Template

Note: The necessary run template (.amxt) file is available from local sales support – depending on your mode of analysis. Alternatively, a run template can be generated from LIMS, please contact us for more information.

1. Turn on Agilent AriaMx™ cycler and the remote computer.
2. Open the Agilent AriaMx™ software and continue via the software.
3. Go to "File", then "Open…".
4. Browse to find the required run template file.

Evaluation sheet only

For evaluation with BAC*Gene* Evaluation Sheet: "BACGene_EV_Salmonella_spp.amxt"

FastFinder and PURE

For evaluation with FastFinder / PURE: "BACGene_Salmonella_spp.amxt"

5. Then click "Open".
6. In the opened experiment tab sample names should be added to the field "Well Name". Alternatively, sample names can be added later during the evaluation of the run.
7. Please check that the following parameters are included in the run template:
   - "Plate Setup" on the right side of the interface under the header "Wells":
     - "Add Dyes":
       - FAM™ and Cy5™ are selected for all relevant 96 wells

- "Target Name"- in order to check target names (table below) click on the arrow next to "Target" on the right side of the screen. If more than one system is analysed on one plate, please use the following target name for respective wells of the BAC*Gene Salmonella* spp. kit and verify the target names for the other assays with the respective kit instruction for use:

BAC*Gene Salmonella* spp.	**Detection Channel**	***Evaluation sheet only*** **Target Name**	***FastFinder and PURE*** **Target Name**
***Salmonella* spp.**	FAM™	2014a	*S.*spp.
IPC	Cy5™	2014i	IPC

Note: The target names have to be correct to ensure the correct evaluation using the BAC*Gene* Evaluation Sheets, FastFinder or PURE software. The correct target names have already been assigned to the wells in run templates provided. For more information, please contact your local sales support.

- Check sample type definitions.

Evaluation sheet only

Sample type definitions for evaluation with BAC*Gene* Evaluation Sheet:

- Well type of the positive control should be defined as "Calibrator" (e.g. well A1)
- Well type of the negative control should be defined as "Buffer" (e.g. well B1)
- Well type of all other wells should be defined as "Unknown"

FastFinder and PURE

Please refer to the short guide 'FastFinder for BAC*Gene* user' or PURE software guide for detailed information

Important: For mixed plates please confirm the parameters using the instructions for use for each of the assays included.

8. Change to the "Thermal Profile" view on the left side of the screen (under "Setup").
9. Confirm cycler protocol

1 HOLD	**42 CYCLES**	
enzyme activation	denaturation	annealing & extension
15 min at 95 °C	15 sec at 95 °C	60 sec at 60 °C
no data collection	no data collection	data collection

Important: The run should always be initiated using a template containing the required information!

10. Select "Run" in the "Thermal Profile" tab to open the "Instrument Explorer".
11. Select the correct AriaMX cycler and click on "Send Config"
12. If required, enter your login details for the selected instrument.
13. Save the experiment under the desired name and in the desired location
14. The run information has now been sent to the selected instrument.
15. The following icon will appear on the bottom right of the screen of the cycler:

16. Click the icon and press “Open Primed Experiment” on the pop-up.

Note: In case “Sync Plate” message appears, please confirm and continue.

17. The experiment will open on the screen of the cycler.
18. Under “Thermal Profile” click “Run Experiment”.

Data Evaluation

After run completion, data should be evaluated using either the BAC*Gene* Evaluation Sheet, FastFinder or PURE software. The data export for the evaluation using the BAC*Gene* Evaluation Sheet is outlined in the instructions tab included in the Evaluation Sheet or in the PURE software guide for PURE evaluation

The evaluation using FastFinder is done using the run files directly without requiring an additional data export, for more details refer to separate short guide “FastFinder for BAC*Gene* kits”.

Note: Please do not use the “Disc Symbol” option on the touch screen for saving runs on a USB stick. Only use the “Copy & paste” option as described in the instrument documentation.

4 BIO-RAD CFX96 TOUCH™: INSTRUCTIONS FOR USE WITH BAC*GENE SALMONELLA* SPP. KIT

The BAC*Gene Salmonella* spp. kit is validated for use with the Bio-Rad CFX96 Touch™ and Bio-Rad CFX96 Touch™ Deep Well with CFX Manager™ software version 3.1/CFX Maestro™ software version 2.3.

The PCR platform should be prepared prior to commencing the practical work. For a more detailed description of the instrument programming please refer to the user manual of the Bio-Rad CFX96 Touch™ or CFX96 Touch™ Deep Well.

BAC*Gene* offers three methods for evaluating the final results. Users can choose between a manual data interpretation using the BAC*Gene* Evaluation Sheet or two automated solutions using FastFinder or PURE Software.

4.1 Running a PCR

The following instructions outline how to set up a PCR run on the Bio-Rad CFX96 Touch™ and Bio-Rad CFX96 Touch™ Deep Well with an external computer.

Note: If you are using the cycler via the touch screen without an external computer please contact your local sales support for more information.

4.1.1 Before You Begin

Note: The required protocol and plate layout templates are available from your local sales support. Furthermore, the CFX Manager™ 3.1 /CFX Maestro™ 2.3 software can be configured for use with a Laboratory Information Management System “LIMS”. Please contact your local sales support for more information.

Note: This procedure only needs to be carried out the first time you are running the assay.

1. Turn on Bio-Rad CFX96 Touch™ (Deep Well).
2. Open the Bio-Rad CFX Manager™ 3.1/CFX Maestro™ 2.3.
3. Go to “File”.
4. Select “Open” and “Protocol”.
5. Browse to select the desired protocol file “BACGene_protocol.prcl” or “BACGene_DW_protocol.prcl” (depending on the instrument used).
6. Click “Open”.
7. In the newly opened window, displaying the protocol selected in step 5, select “File” and then “Save As”.
8. All protocols have to be saved in:
   C:\Users\Public\Documents\Bio-Rad\CFX\Users\admin\ExpressLoad
   Enter “BACGene_protocol” or “BACGene_DW_protocol” as the protocol name in the CFX software, click on “Save” and close the window.
9. Repeat steps 3 – 8 for the respective plate layout template. Depending on the evaluation method (evaluation sheet or FastFinder or PURE software) make sure to import and save the correct plate layout template:

Evaluation sheet only

For evaluation with BAC*Gene* Evaluation Sheet: "BACGene_EV_Salmonella_spp_Plate.pltd"

FastFinder and Pure

For evaluation with FastFinder / PURE: "BACGene_Salmonella_spp_Plate.pltd"

4.1.2 Running a PCR Using a PCR Layout Template

Make sure that the correct protocol and plate layout templates have been imported into the Express Load folder according to section 0. Alternatively, a LIMS template import can be generated directly from LIMS, for more information please contact your local sales support.

1. Open the CFX Manager™ 3.1 or CFX Maestro™ 2.3 software.
2. In the "Startup wizard" select the correct instrument in the "Select instrument" drop-down menu.
3. Under "Select run type" click "User defined"
4. In the "Run setup" window select the BAC*Gene* protocol template from the "Express load" drop-down menu.

Important: The run should always be initiated using the correct protocol and layout templates.

5. Prior to starting the run check that the following parameters are correct:
   - In the "Protocol" tab check:
     - Sample volume: 25 µL
     - Thermal cycler times and temperatures
     - Thermal Profile:

1 HOLD	**41 REPETITIONS**	
enzyme activation	denaturation	annealing & extension
15 min at 95 °C	15 sec at 95 °C	60 sec at 60 °C
no data collection	no data collection	data collection

6. Click on "Next" and open the plate layout template from the "Express load" drop-down menu:

Evaluation sheet only

For evaluation with BAC*Gene* Evaluation Sheet: "BACGene_EV_Salmonella_spp_Plate.pltd"

FastFinder and Pure

For evaluation with FastFinder / PURE: "BACGene_Salmonella_spp_Plate.pltd"

7. Go to "Edit selected..." to open the "Plate editor".
8. Click on "Spreadsheet View/Importer" to add sample names
9. Sample names can be added either manually, via copy and paste or importing a "*.csv" file (click on "Import").

10. After adding sample names, close the spreadsheet view by clicking “OK”.
11. In the “Plate editor” check:
    - Plate Type: BR White
    - Scan Mode: All Channels
    - Fluorophores: FAM™ and Cy5™ are selected for all relevant 96 wells
    - Target Names: To check, click “Edit Selected” and the target names are shown on the right side of the screen, compare with target names in table below. If more than one system is analysed on one plate, please use the following target names for respective wells of the BAC*Gene Salmonella* spp. kit and verify the target names for the other assays with the respective kit instruction for use:

BAC*Gene Salmonella* spp.	**Detection Channel**	***Evaluation sheet only*** **Target Name**	***FastFinder and PURE*** **Target Name**
***Salmonella* spp.**	FAM™	2014a	S.spp.
IPC	Cy5™	2014i	IPC

Note: The target names have to be correct to ensure the correct evaluation using the BAC*Gene* Evaluation Sheet or FastFinder or PURE software. The correct target names are assigned to the wells in templates provided by us. For more information, please contact your local sales support.

- Check sample type definitions:

Evaluation sheet only

Sample type definitions for evaluation with BAC*Gene* Evaluation Sheet:

- Positive control should be defined as “Positive control” (e.g. well A1)
- Negative control should be defined as “Negative control” (e.g. well B1)
- All other wells should be defined as “Unknown”

FastFinder and PURE Please refer to the short guide ‘FastFinder for BACGene user’ or PURE software guide for detailed information

Important: For mixed plates please confirm the parameters using the instructions for use for each of the assays included.

12. Click “OK” to close the “Plate Editor” and select “Next” in the “Run setup” tab to get to the “Start Run” tab.
13. Select the cycler from the list.
14. Click “Open Lid”, load the plate into the cycler and then press “Close Lid”.
15. Click “Start Run”.
16. In the directory, choose the location to save the run file and then click “Save”.

4.2 Data Evaluation

After run completion, data should be evaluated using either the BAC*Gene* Evaluation Sheet, FastFinder or PURE software. The data export for the evaluation using the BAC*Gene* Evaluation Sheet is outlined in the instructions tab included in the Evaluation Sheet or in the PURE software guide for PURE evaluation

The evaluation using FastFinder is done using the run files directly without requiring an additional data export, for more details refer to separate short guide “FastFinder for BAC*Gene* kits”.

5 DATA INTERPRETATION

Data is interpreted using the 'BAC*Gene* Evaluation Sheet' ,FastFinder or PURE software .

For an evaluation using the evaluation sheet upload data as described in 'BAC*Gene* Evaluation Sheet' file.

For FastFinder or PURE software please refer to the "FastFinder for BAC*Gene* kits" instruction for use or the PURE software guide.

Interpretation of final sample results is summarised in the following table:

Salmonella	**Internal Positive Control**	**Final results**
Reaction positive	Not relevant	Positive for *Salmonella* spp.
Reaction negative	Valid	Negative for *Salmonella* spp.
Reaction negative	Invalid	Questionable*

** Refer to troubleshooting section (see chapter 0).*

6 CONFIRMATION OF PRESUMPTIVE POSITIVE RESULTS

In the context of NF VALIDATION and AOAC-PTM certification, all samples identified as positive by the BAC*Gene Salmonella* spp. kit must be confirmed by (one of) the following test(s):

Option 1:

Using the conventional tests described in the methods standardized by CEN or ISO from colonies (including the purification step). The confirmation step must start from the enrichment broth.

Option 2:

By subculture of 100 µL from the enrichment broth in 9.9 mL RVS (incubation 21 –27 h at 41.5°C ± 1°C) followed by streaking onto XLD followed by latex test (i.e. Oxoid) directly on isolated typical colonies without a purification step.

Option 3:

Using any other method certified according to NF VALIDATION or AOAC, respectively, the principle of which must be different from the BAC*Gene Salmonella* spp. kit. The protocol of detection of the second validated method used for the confirmation shall be followed entirely. All steps which are before the step from which the confirmation is started shall be common to both methods. The BAC*Gene Salmonella* spp. kit and the second validated method must have common first steps, for instance the same enrichment broth.

Independent of the confirmation method used, in the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above, in particular by the latex test) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

7 TROUBLESHOOTING

BAC*Gene* offers three methods for evaluating the final results. Users can choose between the manual interpretation using the BAC*Gene* Evaluation Sheet,FastFinder or PURE software for an automated evaluation. Evaluation specific troubleshooting comments can also be found in the respective user guide for FastFinder or PURE. (marked as the colored boxes below)

Evaluation sheet only

FastFinder only

7.1 Oberservations upon Delivery/During Storage and Reception

Observation:

MasterMix plate is not frozen upon arrival and without appropriate cooling (dry ice)

Possible Cause	Solution
Plates defrosted during shipping	Contact your local sales support or your local distributor.

Observation:

MasterMix was thawed

Possible Cause	Solution
Freezer problems	Install an external alarm system on your freezers in order to get notice if there are problems. Gold Standard Diagnostics takes no liability for products stored incorrectly. If you nevertheless would like to test the performance of the product, contact your local sales support for guidelines.
MasterMix was not stored at –20 °C	Gold Standard Diagnostics takes no liability for products stored incorrectly. If you nevertheless would like to test the performance of the product, contact your local sales support for guidelines.

7.2 Observations Associated with Handling of the Plates

Observation:

Frozen MasterMix film covering the well

Possible Cause	Solution
Plates were not thawed and spun down before removing the seal.	Use fresh strips, thaw and spin down before removing the seal.

Observation:

Liquid MasterMix film covering the well

Possible Cause	Solution
Plates were not spun down after thawing and prior to removing the seal.	Use fresh strips and spin down before opening the seal.

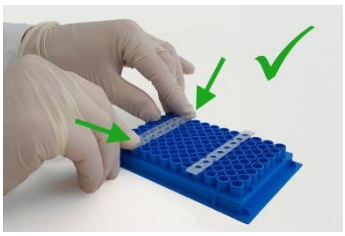
Observation:

MasterMix is spilling during the removal of the seal

Possible Cause	Solution
MasterMix plate is not fixed appropriately within the PCR rack.	Make sure to fix the strip with one hand and remove seal strictly in 180-degree angle (not pulling upwards, but towards yourself). Review instructions in section 2.7.4

Observation:

Optical cap strips do not align with wells and plastic is damaged

Possible Cause	Solution
The strip was closed in one direction.	To facilitate closing the cap strips on PCR strips first softly fix the ends of the cap strip to the reaction tubes, then apply even pressure to tightly close the PCR strips.  

7.3 Incorrect Data Export and Import to BAC*Gene* Evaluation Sheet

Evaluation sheet only

Observation:

Data import into the BACGene Evaluation Sheet was not successful/possible

Possible Cause	Solution
The content has not been enabled and Macros are not running properly.	Ensure the content is enabled by following instructions in links provided in the "BAC*Gene* Evaluation Sheet" or on the official Microsoft Office website (http://office.microsoft.com/). Then repeat the evaluation.
Data files are not recognized for the import.	Agilent AriaMx™: Ensure that "Text" is selected as file type in the "Export Data" section. Bio-Rad CFX96 Touch™ (Deep Well): Ensure that "Text" (as Export Format) and "Tab" (as Column Separator) are selected in the "Custom Export" section.
Relevant data is missing in the export files.	Ensure that all export files are saved in the same folder and the file names are not changed during/after export. When prompted, manually chose the respective files or repeat the export. Agilent AriaMx™: Ensure that all relevant fluorophores/targets are selected in the "Graphical Displays" section for all relevant wells. Ensure that all columns are selected for export in "Tabular results" file (see section 0). Bio-Rad CFX96 Touch™ (Deep Well): Ensure that all relevant fluorophores/targets are selected for all relevant wells in the "Plate Editor" section. Ensure that the correct columns were selected during the "Custom Export" from the CFX software (see section 0).

7.4 Incorrect Run File Upload Using FastFinder Software

Observation:

The samples are not automatically labelled (sample definition and/or assay type) on the plate layout in the FastFinder analysis

Possible Cause	Solution
Run file templates are not used or nametags are not correctly applied	Use the templates provided to correctly label your samples and controls automatically. If you are not using the templates check if the sample type definitions and target names (nametags) are correctly assigned for the use with FastFinder (section 0 and 4.1.2). Sample type definitions and target assays can also be assigned manually in FastFinder by dragging and dropping sample definitions to the respective wells or selecting the wells to be edited and choosing the correct sample definitions.

7.5 Inconclusive Results Post-Evaluation

Observation:

No positive/negative controls set. Evaluation is not possible

Possible Cause	Solution
C+ or C- have not been assigned.	***Evaluation sheet only*** Go to the "Results tab view" worksheet and define the correct task (C+ and/or C-) via the "Task" drop-down menu (Column G of the Evaluation Sheet) for the corresponding wells. Alternatively: Define the respective Tasks in the original run file and repeat the export and evaluation. ***FastFinder and PURE*** Restart analysis and identify/select correct location of C+ and C-.

Observation:

The negative control (C-) is positive for at least one target assay

Possible Cause	Solution
Cross contamination occurred.	Follow decontamination procedure. Repeat lysis of enrichment and repeat PCR using fresh aliquots of all reagents. If C- continues to show a positive signal, contact your local sales support.
C- has not been assigned correctly.	Make sure that the correct well for C- is selected.

Observation:

C- not in range (in the IPC system) – evaluation not possible

Evaluation of the IPC is dependent on the C- control reaction. If this reaction is not in range, the IPC cannot be evaluated in the sample reactions.

Possible Cause	Solution
MasterMix plate was freeze-thawed more than 3 times	The MasterMix plate should not be frozen and thawed more than 3 times as it may cause inactivation of the reagents.
95°C lysis step was not performed correctly.	Ensure the heating block reaches the correct temperature (95 °C ± 5 °C) by measuring with a thermometer. Ensure this step is performed for the correct duration (10 min).
The MasterMix was not in the bottom of the PCR tube, but in the lid.	Always visually check the level of the MasterMix before use. If the MasterMix is not at the bottom of the tubes, spin down before use.
C- has not been assigned correctly.	Make sure that the correct well for C- is selected.
Automated threshold calculation failed.	***Evaluation sheet only*** Refer to section 7.6.

Observation

C+ is negative/too late in the required target system - evaluation is not possible.

Note: The warning message „Late Cq value of C+“ is an indication reduced performance of the C+. However, evaluation of the results is still valid. Also review troubleshooting recommendations below.

Possible Cause	Solution
Pipetting error - no positive control or too little positive control material was added.	Repeat PCR ensuring 5 µL of the positive control material is pipetted into appropriate well (A1).
The positive control was not stored correctly.	If incorrect storage is suspected, use a fresh tube of positive control material. When starting a new kit, always use the positive control material from that kit.
The positive control material has undergone too many freeze-thaw cycles.	Use a fresh tube of positive control material. Do not freeze/thaw more than 6 times. If C+ continues to show a negative signal, contact your local sales support.
The frozen master mix was not in the bottom of the PCR tube, but in the lid.	Always visually check the level of the master mix before use. If the master mix is not at the bottom of the tubes, allow to thaw, spin down and re-freeze before use.
C+ has not been assigned correctly.	Make sure that the correct well for C+ is selected. Additionally, be sure the correct target name is selected on the cycler software.
Automated threshold calculation failed.	***Evaluation sheet only*** Refer to section **Hiba! A hivatkozási forrás nem található.**.

Evaluation sheet only

Observation

Cq value of the C+ is too early (in the required target system) – evaluation is not possible.

Possible Cause	Solution
C+ has not been assigned correctly.	Make sure that the correct well for C+ is selected.
Automated threshold calculation failed.	Refer to section **Hiba! A hivatkozási forrás nem található.**.

Observation

A "Q" or invalid result is observed for sample

Possible Cause	Solution
Missing PCR tubes.	The analysed well might actually be empty. Check layout of tubes in PCR platform to ensure it matches the analysed layout.
The frozen master mix was not in the bottom of the PCR tube, but in the lid.	Always visually check the level of the master mix before use. If the master mix is not at the bottom of the tubes, allow to thaw, spin down and re-freeze before use.
95 °C lysis step was not performed correctly.	Confirm that the heating block reaches the correct temperature (95 °C ± 5 °C) by measuring with a thermometer. Repeat lysis of enrichment and repeat the PCR.
Sample lysate was not taken from upper half of tube when transferring to PCR.	Repeat lysis of enrichment and repeat PCR. Ensure to take sample from upper half of tube.
Inhibition of PCR or inconclusive results (refer to "Data interpretation" table, section 5).	**Note:** Leaving the enrichment bag or aliquot in sterile container to rest for 5 – 10 minutes after agitation and before lysis to allow sedimentation of crude suspended particles might reduce the inhibitory effect of some matrices. If PCR remains inhibited, repeat lysis of enrichment and repeat PCR. If PCR remains inhibited, dilute the lysate sample with DNA-/Nuclease free water (one-part sample plus four parts water and one-part sample plus nine parts water). If inhibition still persists, review instructions of the respective target specific reference method and the ISO 6887 series for preparation of initial suspensions. Check if the matrix type has been validated for use with the BAC*Gene* kit. The method should only be applied to validated matrices.
Automated threshold calculation failed.	***Evaluation sheet only*** Refer to section **Hiba! A hivatkozási forrás nem található.**.

7.6 Incorrect Automated Threshold Settings in Cycler Software

Evaluation sheet only

Important: Before manually adjusting any settings, please make sure that all steps described in the data export section 3.2 (for AriaMx™) and 4.2 (for Bio-Rad CFX96 Touch™ Standard and Deep Well) were followed.

The interpretation of real-time PCR runs depends on automated algorithms that analyse and interpret the measured fluorescence data.

These algorithms are thoroughly validated by the manufacturers of the PCR cyclers and the respective evaluation software and provide reliable results for the vast majority of analyses.

In rare cases, when the automated threshold calculation of the cycler software fails, manual adjustments are reasonable and justified in order to get a correct interpretation of the measured data (e.g. automatic threshold settings might calculate a threshold which is not set in the exponential phase of the amplification curves).

Examples of threshold settings for Agilent AriaMx™ and Bio-Rad CFX™:

In both software platforms the threshold can be adjusted via drag & drop if the automatic threshold calculation fails.

In case of failure of the automatic threshold settings, it is possible to review the curves and set the threshold within the exponential phase (phase 2; Figure 2 and Figure 3) of the amplification curve (signal intensity doubling in each cycle) before it gets into a phase with steady linear increase of the signal intensity (phase 3; Figure 2 and Figure 3).

Note: The adjustment is described for the linear view. For AriaMx™ make sure Graph Type “Linear” is selected. For Bio-Rad CFX™ ensure that the box “Log Scale” is unchecked.

Agilent AriaMx™ software:

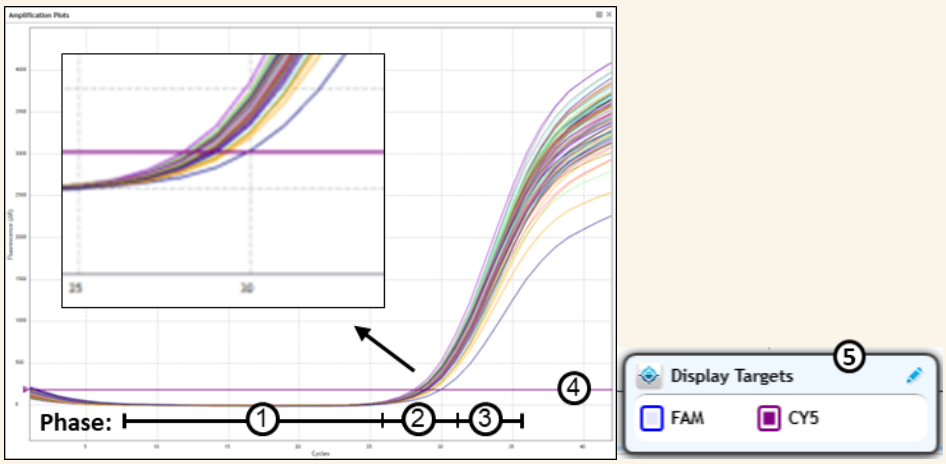


Figure 2. Exemplary amplification plot of a real-time PCR in linear scale (Graph Type “Linear”). 1, background; 2, exponential amplification phase; 3, linear amplification phase; 4, threshold line; 5, displayed target, be sure to set threshold for each target/detection channel individually.

Bio-Rad CFX™ software:

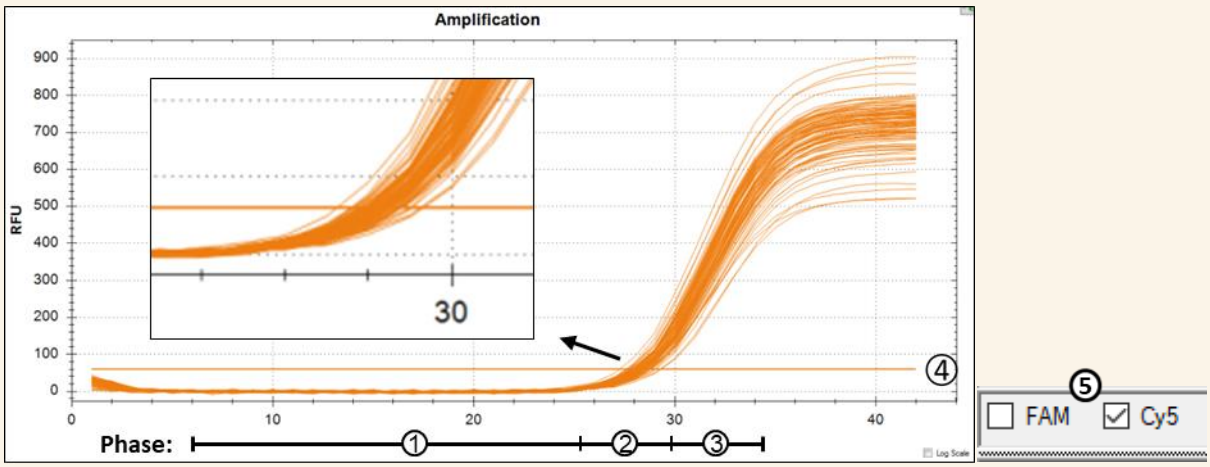


Figure 3. Exemplary amplification plot of a real-time PCR in linear scale (Box "Log Scale" is unchecked). 1, background; 2, exponential amplification phase; 3, linear amplification phase; 4, threshold line; 5, displayed target, be sure to set threshold for each target/detection channel individually.

7.7 Cultural Confirmation

Observation:

The positive result in the Salmonella PCR assay(s) cannot be confirmed by culture (no isolated typical colonies).

Possible Cause	Solution
Pre-enrichment bag was not mixed thoroughly before taking the portion to streak onto selective agar plates.	Always mix the pre-enrichment thoroughly (as target organisms can settle) before taking out the portion to streak onto selective agar plates.
The volume streaked onto the selective agar plates was not sufficient.	Always make sure the loop for streaking is completely covered with 10 µL of the culture, if this does not work, use 50µL of the culture for plating.
Quality of selective agar plates is not sufficient.	Make sure agar plates are quality tested (e.g. according to ISO 11133).
Target organism cannot be detected on plates as they are overgrown with background flora.	Make sure the selective agar plates are prepared and stored according to the manufacturer's instructions and quality-controlled (e.g. according to ISO 11133).
Contamination with genomic (free DNA from dead bacteria) in the environment causes positive PCR results.	Make sure all recommendations regarding DNA contamination prevention are followed and take measures to identify the source of a possible contamination (i.e. environmental swabbing for DNA contamination and/or block contamination test for Agilent AriaMx).
Free DNA from dead target organism cell in the sample produces a positive PCR result.	General: Use of PR*Eraser* BAC*Gene* is recommended for samples in which a high level of free DNA from dead cells is expected. PR*Eraser* is applied before lysis of the living cells in order to eliminate signals from free DNA. Refer to the PR*Eraser* BAC*Gene* kit instruction for use and relevant certifications for details about the scope and applicability of the method for the matrix in question. Environmental samples: Environmental samples should be taken in a way that reduces sampling of dead bacteria, e.g. by rinsing of surfaces after decontamination to remove dead bacteria.

8 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

Gold Standard Diagnostics Budapest ("GSDB") warrants the products manufactured by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions before the expiration date marked on the product packaging and when stored under the storage conditions recommended in the instructions and/or on the package. GSDB makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

GSDB's sole obligation with the respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies GSDB promptly of any such defect. GSDB shall not be liable for any direct, indirect or consequential damages resulting from economic loss or property damages sustained by buyer or any customer from the use of the product(s). A copy of Gold Standard Diagnostics Budapest terms and conditions can be obtained on request, and is also provided in our price lists.

9 LIMITATIONS FOR USE

The BAC*Gene Salmonella* spp. kit is designed to detect DNA from target organisms as specified when grown to detectable levels during enrichment.

The BAC*Gene Salmonella* spp. kit has been tested broadly against many, but not all strains. Following AFNOR NF and AOAC PTM guidelines we ensure high level of inclusivity. However, given the nature of DNA-based molecular detection technologies, Gold Standard Diagnostics cannot make any representation or warranty that BAC*Gene Salmonella* spp. is capable to detect every single strain or mutants of the genus. Accordingly, BAC*Gene Salmonella* spp. should not be used as the sole test for the release of user's products, nor should it be used as the sole bases for determining the safety of user's products.

The BAC*Gene Salmonella* spp. kit has been tested using a vast variety of matrices following AFNOR NF and AOAC PTM guidelines. Given the variety and composition of semi-finished or finished products within the different food categories, it is mandatory to verify or validate food matrices to avoid any impact on the reliability of the BAC*Gene* results.

The BAC*Gene Salmonella* spp. kit shall not be used in conjunction with environmental swabs, pre-moistured with neutralizing buffers containing aryl sulfonate (sodium dodecylbenzenesulfonate).

10 IMPORTANT NOTES

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11 VERSION CONTROL

Version	Date	Changes
V4	xxx	• Update of AriaMX and CFX Software Versions • Inclusion of PURE as evaluation software • Inclusion of the use of the OT-2 automation system for BAC*Gene* use
V3.11	30.05.2023	• Inclusion “Introduction” section • Inclusion “Limitations for Use” section • Clarified PREraser sections • No product or assay-specific changes
V3.10	06.12.2022	• Adaptation to Gold Standard Diagnostics layout for BAC*Gene* kits • Change of contact address • Deletion of the section "Product Use Limitations" • Add section "Version Control" • Change of file name • Adjustment of structuring in some sections • Remove/correct some catalogue numbers • Change the section “Cleaning Protocol” • No product or assay-specific changes

TECHNICAL SUPPORT SERVICE

For technical assistance and more information, please contact Gold Standard Diagnostics Budapest's customer service or your local distributor.

Gold Standard Diagnostics Budapest Kft.
Fóti út 56 A ép.
1047 Budapest, Hungary

www.goldstandarddiagnostics.com