

# **BACGene Salmonella spp.**

**Test kit for qualitative real-time PCR detection of  
*Salmonella* spp.**

Cat. No. 5123221801:

96 lysis and real-time PCR reactions

Cat. No. 5123221810 (10x high throughput (HTP) kit):

10 x 96 lysis and real-time PCR reactions

Cat. No. 5123221811 (10x content of cat. no. -01):

10 x 96 lysis and real-time PCR reactions



EGS 38/01-03/15  
ALTERNATIVE ANALYTICAL  
METHODS FOR AGRIBUSINESS  
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## 1 INTRODUCTION

The BACGene *Salmonella* spp. detection kit provides materials for rapid detection of *Salmonella* spp. DNA from food, feed (including pet food) and environmental samples.

The BACGene *Salmonella* spp. detection kit may also be useful for other purposes in food product research and analysis field, e.g. microbial monitoring of production processes.

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

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

### 1.1 Certifications

#### 1.1.1 AFNOR Certification

The BACGene *Salmonella* spp. kit is certified by AFNOR Certification as an alternative method for *Salmonella* spp. detection in 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)
- Environmental samples



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

### 1.1.2 AOAC Performance Testing

The BACGene *Salmonella* spp. kit is certified by AOAC-Research Institute under the Performance Tested Methods<sup>SM</sup> 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 *PREraser*\*)
- 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 *PREraser*\*)
- 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 *PREraser*\*)
- 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.

\**PREraser* BACGene (see sections 1.5.1 and 2.5.1) has been performance tested for three relevant matrices. Therefore, provided that additional matrices are verified, *PREraser* BACGene can be applied to other matrices within the scope of the method.

## 1.2 Important Notes

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

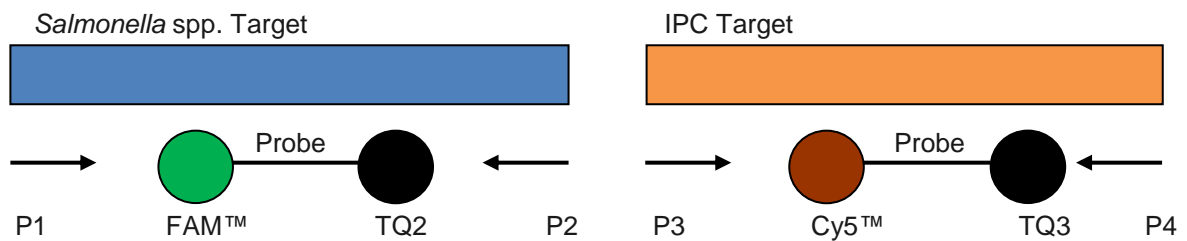
## 1.3 Test Principle

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 labels of the components.

#### **For Lysis:** Cat. No. 5123221801 / 5123221811

1x/10x	<b>Lysis plate for sample preparation,</b> empty, rippable (high profile)
1x/10x	<b>Domed caps*</b> , for use with Lysis plate, set of 12 strips
2x/20x	<b>Lysis buffer I,</b> 4 mL, store at -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/20x	<b>Proteinase K,</b> vials with blue cap, each with 500 µL, store at -20°C ± 2°C

#### **For Lysis:** Cat. No. 5123221810 (10x HTP)

10x	<b>Lysis plate for sample preparation,</b> empty, rippable (high profile)
10x	<b>Domed caps*</b> , for use with lysis plate, set of 12 strips
2x	<b>Lysis buffer I,</b> 40 mL, store at -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	<b>Proteinase K,</b> 5 mL, store at -20°C ± 2°C

\*Note: additional domed caps for lysis in a package of 250 x 8 cap strips with Cat no. 5613900301 can be purchased at [kits@eurofins.com](mailto:kits@eurofins.com).

#### **For PCR:** Cat. No. 5123221801 / 5123221810 (1x/10x)

1x/10x/10x	<b>BACGene <i>Salmonella</i> spp. PCR plate,</b> with pre-dispensed MasterMix and PCR support grid. Store light protected at -20°C ± 2°C.
1x/10x/10x	<b>Optical caps,</b> for use with PCR plate. Mat of 12 strips, rippable.
2x/4x/20x	<b><i>Salmonella</i> positive control plasmid DNA,</b> vial with yellow cap, 50 µL, store at -20°C ± 2°C. Do not freeze/thaw more than 6 times.

## 1.5 Additional Equipment, Consumables and Reagents Required

### 1.5.1 Sampling / Enrichment

- Neutralizing buffer, e.g.
  - Dey-Engley
  - Lethen broth
  - HiCap broth
- Swabs / sponges / wipes / cloths (for environmental samples)\*
  - Eurofins Technologies EZ Reach™ Sponge Sampler, Polyurethane, 10 mL HiCap Neutralizing Broth, 24 oz bag, 100 pcs, cat. no. EUR EZ-10HC-PUR
- MRB medium (for carcass sampling)
- Diluents according to EN ISO 6887-1 (for carcass sampling)
- Stomacher® or paddle blender, capable of running between 15.000 and 20.000 rev/min
- Enrichment bags with side filter
- 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® (for products containing >20 % fat, including cocoa containing products, unless the products already contain sufficient emulsifier).
  - Add sufficient amount of polysorbate 80 [polyoxyethylene (20) sorbitan monooleate] 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 6884-4:2017).
- PR*Eraser* BACGene, Eurofins GeneScan Technologies, cat. no. 5123222301 or 5123222311
  - For the sample pre-treatment after enrichment and prior to lysis.

**\*Note:** Products pre-moistened with neutralizing buffers containing aryl sulfonate (sodium dodecylbenzenesulfonate) should not be used in conjunction with this kit. Recommended is the use of Dey-Engley neutralizing buffer, Lethen broth or HiCap broth.



## 1.5.2 Lysis

### Equipment

- 1x 96 well cooling block (e.g. cat. no. 5613900501)
- 2x Heating block 96 well, 0.2 mL  
(e.g. cat. no. 5613900701 for 37°C, cat. no. 5613900801 for 95°C)
- 2x Insert for heating block 0.2 mL wells  
(e.g. cat. no. 5613900901)
- Blue work rack for lysis  
(e.g. cat. no. 5613901101)
- Strip centrifuge :
  - Capacity of 2x 8-well strips: (e.g. Carl Roth GmbH, Rotilabo<sup>®</sup> centrifuge with butterfly rotor cat. no. T465.1).
  - Capacity of 4x 8-well strips: (e.g. Mini Centrifuge IKA Mini G, cat. no. 5613902601 or VWR, MiniStar silverline cat. no. 521-2844P).

or

Plate centrifuge:

- Capacity of two times 12x 8-well strips: (e.g. Benchmark Scientific, PlateFuge<sup>™</sup> microplate microcentrifuge, cat. no. 5613901701).
- Capping/Uncapping tool for 8-well strips (e.g. MicroAmp<sup>®</sup> Cap Installing Tool, cat. no. 5613900401)
- Stepper pipette (1 mL) (e.g. HandyStep<sup>®</sup> S (Brand<sup>®</sup>), cat. no. 5617703401)
- Single channel pipettes (1 mL, 100 µL)  
(e.g. Transferpette<sup>®</sup> S 100 - 1000 µL (Brand<sup>®</sup>), ca. no. 5617703301)
- Pipette filler for serological pipettes

### Consumables

- DNA-/Nuclease-free pipette tips
- 1 mL stepper tips
- Sterile container for enrichment storage
- Serological pipettes (e.g. cat. no. 5617702405 (1 mL) or cat. no. 5617702505 (10 mL))
- Powder free gloves

### 1.5.3 PCR Setup

#### Equipment

- Pink work rack (e.g. cat. no. 5613901201)
  - Capping/Uncapping tool for 8-well strips (e.g. MicroAmp<sup>®</sup> Cap Installing Tool, cat. no. 5613900401)
  - Multichannel pipette (5 µL) (e.g. Transferpette<sup>®</sup> S-8 Kanal / 0.5 - 10 µL (Brand<sup>®</sup>), cat. no. 5617703501)
  - Single channel pipettes (5 µL) (e.g. Transferpette<sup>®</sup> S, 0.5 - 10 µL (Brand<sup>®</sup>), ca. no. 5617703101)
  - Disposable transfer rack (e.g. an empty tip box) as mount for the yellow support grid and the PCR plate
  - Real-time PCR Thermocycler:
    - Agilent AriaMx<sup>™</sup> (cat no.: G8830A) (up to Software v.1.5), with FAM<sup>™</sup> and Cy5<sup>™</sup> filter set.
    - Bio-Rad CFX96 Touch<sup>™</sup> (CFX Manager<sup>™</sup> Software v. 3.1 / CFX Maestro<sup>™</sup> Software 1.1).
    - Bio-Rad CFX96 Touch<sup>™</sup> Deep Well (CFX Manager<sup>™</sup> Software v. 3.1 / CFX Maestro<sup>™</sup> Software 1.1).
  - Windows 7/10 PC with Microsoft Excel 2010 32 bit (Excel Version 14.7140.xxxx or later).
  - BACGene Evaluation Sheet version 2
- or
- FastFinder automated PCR analysis software (UgenTec NV, Hasselt, Belgium) with BACGene *Salmonella* spp. plugins with version 1

#### Consumables

- Roti<sup>®</sup> Nucleic Acid-free (Carl Roth GmbH, cat. no. HP69) or 1% HCl (for DNA decontamination)
- DNA-/Nuclease-free pipette tips
- Nuclease free water (molecular biology grade)
- Powder free gloves

## 2 HOW TO USE THIS PRODUCT

### 2.1 Safety Precautions

- All samples should be handled with caution as they are potentially infectious.
- Do not eat, drink or apply cosmetics in the work area where 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 immunocompromised 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 BACGene *Salmonella* spp. kit contains proteinase K which may cause allergic reactions (EUH 208).

The BACGene *Salmonella* spp kit contains no hazardous components.

For more information, please refer to the BACGene *Salmonella* spp kits safety data sheet.

### 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
- 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 labware
- Wear gloves
- Change gloves after removing samples from the enrichment, before starting the lysis protocol

### 2.2.1 Cleaning protocol

For use of this kit, two different types of contamination can impair results: Bacterial contamination is prevented by regular disinfection, DNA contamination is prevented by regular decontamination.

Before commencing work and after completion of work, ensure workspaces to be clean:

Workspace	Cleaning protocol
Enrichment preparation area	Disinfect surfaces with 80% ethanol.
Lysis area	Disinfect surfaces with 80% ethanol. Decontaminate surface with Roti® Nucleic Acid-free or 1% HCl. Pipettes used during lysis should also be decontaminated with Roti® Nucleic Acid-free.
PCR area	Decontaminate surfaces with Roti® Nucleic Acid-free or 1% HCl.

### 2.3 Waste Disposal

Dispose 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 homogenize 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 enrichment controls (positive and negative) in parallel with the samples. Growth in the negative enrichment control indicates contamination of the medium used and therefore enrichment should be repeated.

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

Certified categories by AFNOR (EGS 38/01-03/15)					
Sample Category	Enrichment			Volume for Analysis	
	Medium	Temp	Time	Enrichment	Lysate
All human food products (except Milk & Dairy) <b>25 g</b>	1:10 BPW	37 ± 1°C	16 - 24 h	10 µL	5 µL
Feed products (incl. pet food)* <b>25 g</b>					
Dusts, process water <b>25 g / mL</b>					
Swab <b>1</b>	10 mL BPW				
Wipe, cloth <b>1</b>	225 mL BPW				
Sponge <b>1</b>	100 mL BPW				
Milk powders & infant formula without probiotics <b>up to 375 g</b>	1:10 BPW (pre-warmed to 37 ± 1°C)		18 - 24 h		
Milk powders & infant formula containing probiotics <b>up to 375 g</b>	1:10 double strength BPW (pre-warmed to 37 ± 1°C)				
Pet food <b>up to 375 g</b>	1:10 BPW (pre-warmed to 37 ± 1°C)				
Milk & Dairy <b>25 g / mL</b>	1:10 BPW		41.5 ± 1°C		
<b>*Note:</b> Storage at 2-8°C for up to 72h is not certified for this category					

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 <b>25 g</b>	1:10 BPW	37 ± 1°C	16 - 24 h	10 µL	5 µL
Dry dog pellets <b>25 g</b>			18 - 24 h		
Process water from scalding tank <b>25 mL</b>					
Ice cream with chocolate inclusions <b>25 g</b> (incl. <i>PREraser</i> )					
Pet food dry pellets <b>375 g</b> (incl. <i>PREraser</i> )					
Stainless steel environmental surface <b>1" x 1" area</b>	10 mL BPW	37 ± 1°C	18 - 24 h	10 µL	5 µL
Ceramic tiles environmental samples <b>4" x 4" area with sponge</b> (incl. <i>PREraser</i> )	100 mL BPW				
Infant formula milk powder supplemented with probiotics ( <i>Bifidus lactis</i> ) <b>up to 375 g</b>	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) <b>up to 375 g</b>	1:10 BPW (pre-warmed to 37 ± 1°C)				
Raw whole milk <b>25 mL</b>	1:10 BPW				

<b>Certified matrices by AOAC (License No: 121501): Rapid protocols of selected meat matrices &amp; carcass cloths</b>					
Matrix	Enrichment			Volume for Analysis	
	Medium	Temp	Time	Enrichment	Lysate
Ground beef <b>up to 375 g</b>	1:10 BPW (pre-warmed to 41.5 ± 1°C)	41.5 ± 1°C	10 – 18 h	50 µL	5 µL
Ground pork <b>up to 250 g</b>					
Pork carcass cloths Pre-moistened with MRB <b>1 cloth</b>	90 mL BPW (pre-warmed to 41.5 ± 1°C)				
Marinated chicken with brine <b>up to 250 g</b>	1:10 BPW (pre-warmed to 37 ± 1°C)	37 ± 1°C			
Pork carcass cloths Pre-moistened with diluents (according to 6887-1) <b>1 cloth</b>	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	Enrichment	Lysate
Cocoa powder Cocoa liquor Milk chocolate Cocoa butter Cocoa crumb <b>up to 375 g</b>	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 (RT*)	41.5 ± 1°C	3 - 5 h	10 µL (of 2 <sup>nd</sup> enrichment)	5 µL

\*RT = 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 skimmed / non-fat milk (0.1 – 0.3% fat) by Buffered Peptone Water results in a significant increase in *Salmonella* growth for these matrix items.

Incubation times (18 - 24h) 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.



## 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

Cat no. 5123221801 (1x):

Add 500 µL proteinase K (vial with blue cap) to 4 mL lysis buffer I (transparent container).

Cat no. 5123221810 (10x):

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 blue working 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 complete 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.

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 *PREraser* BACGene

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.

*PREraser* BACGene 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 BACGene PCR to exclude the presence of free DNA.

Please refer to the *PREraser* BACGene user's manual to include *PREraser* BACGene to the workflow.

*PREraser* BACGene is not part of the AFNOR certified workflow for *Salmonella* spp.

See section 1.5.1 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 (± 2°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 2000 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 BACGene *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.

#### Test samples:

20 µL MasterMix + 5 µL lysate per reaction

#### Positive control (C+):

20 µL MasterMix + 5 µL *Salmonella* positive control plasmid DNA

#### Negative control (C-):

20 µL MasterMix + 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

\* It is recommended to use a *PREraser* negative control (C- or E- with *PREraser*) 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 master mix, necessary for testing the amount of samples to be analysed and store the remainder at  $-20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ .

**Note:** Frequent freezing and thawing might cause inactivation of the reagents. Do not freeze/thaw more than 3 times.

- Thaw master mix and spin down the strips for 30 sec between  $400 - 2000 \times g$ .
  - Visually check that all master mix 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  $5\text{ }\mu\text{L}$  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 master mix.

6. Add  $5\text{ }\mu\text{L}$  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. Transfer the PCR plate to the real-time instrument and start the run (refer to section 3 for Agilent AriaMx™ + section 4 for Bio-Rad CFX96 Touch™).
11. Store the lysis plate at  $-20 \pm 2\text{ }^{\circ}\text{C}$  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 tightly close the PCR strips.

### 3 AGILENT ARIAMX™: INSTRUCTIONS FOR USE WITH BACGENE SALMONELLA SPP. KIT

The BACGene *Salmonella* spp. kit is validated for the use on the Agilent AriaMx™ with software versions up to 1.5.

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™.

BACGene offers two methods for evaluating the final results. Users can choose between the automated evaluation software FastFinder or the BACGene Evaluation Sheet. Evaluation specific comments are marked in the protocol below in colored boxes:

*Evaluation sheet only*

*FastFinder only*

#### 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 us at [kits@eurofins.com](mailto:kits@eurofins.com) for more information.

**Note:** The Aria software is able to interface with a Laboratory Information Management System “LIMS”. Please contact [kits@eurofins.com](mailto:kits@eurofins.com) for more information.

##### 3.1.1 Running PCR Using a Template

**Note:** The necessary run template (.amxt) file is available via [kits@eurofins.com](mailto:kits@eurofins.com). Alternatively a run template can be generated from LIMS, please contact us for more information.

12. Turn on Agilent AriaMx™ cycler and the remote computer.
13. Open the Agilent AriaMx™ software and continue via the software:
14. Go to “File”, then “Open...”
15. Browse to find the required run template file.

*Evaluation sheet only*

For evaluation with BACGene Evaluation Sheet: “BACGene\_EV\_Salmonella\_spp.amxt”

*FastFinder only*

For evaluation with FastFinder: “BACGene\_FF\_Salmonella\_spp.amxt”

16. Then click “Open”.
17. 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.
18. 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 BACGene Salmonella spp. kit and verify the target names for the other assays with the respective assay manual:

BACGene Salmonella spp.	Detection Channel	<i>Evaluation sheet only</i>	<i>FastFinder only</i>
		Target Name	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 BACGene Evaluation Sheets or FastFinder software. The correct target names have already been assigned to the wells in run templates provided. For more information please contact us via [kits@eurofins.com](mailto:kits@eurofins.com).

- Check sample type definitions

*Evaluation sheet only*

Sample type definitions for evaluation with BACGene 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 only*

Sample type definitions for evaluation with FastFinder software:

- Well name of positive control should start with "C+" (e.g. well A1)
- Well name of negative control should start with "C-" (e.g. well B1)

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

19. Change to the "Thermal Profile" view on the left side of the screen (under "Setup").

20. Confirm cyclor 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!

21. Select "Run" in the "Thermal Profile" tab to open the "Instrument Explorer".

22. Select the correct AriaMX cyclor and click on "Send Config"

23. If required, enter your login details for the selected instrument.

24. Save the experiment under the desired name and in the desired location.



**FastFinder only**

**Note:** For the use with FastFinder save run files in the folder defined as “To be processed” in the software (see separate short guide “FastFinder for BACGene kits”).

25. The run information has now been sent to the selected instrument.
26. The following icon will appear on the bottom right of the screen of the cyclor:



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

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

28. The experiment will open on the screen of the cyclor.
29. Under “Thermal Profile” click “Run Experiment”.

### 3.2 Data evaluation

After run completion, data should be evaluated using either the BACGene Evaluation Sheet or the FastFinder software. The data export for the evaluation using the BACGene Evaluation Sheet is outlined below.

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 BACGene kits”.

**Evaluation sheet only**

#### Export of Data for the Evaluation Using the BACGene Evaluation Sheet

1. Open Agilent AriaMx™ software (up to v 1.5) on your PC and open the run file by going to “File”, “Open...” and browsing to the correct location.

**Note:** If you are using the cyclor via the touch screen without an external computer please contact us at kits@eurofins.com for more information.

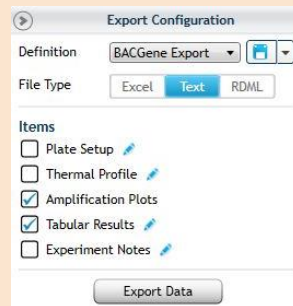
2. Ensure all relevant wells are selected in the “Plate Setup” screen.
3. As a final check before export go to “Graphical Displays” under the “Analysis” header and ensure that the “Background Based Threshold” is set to Cycle Range “5 thru 9” and “Sigma Multiplier 10”. This option can be found by clicking the arrow next to “Amplification Plots” on the right side of the software interface.



4. To prepare a default configuration for result export, follow these instructions. This step only needs to be carried out the first time you use the software. If the default configurations have already been selected continue to step 5.
  - Go to “Export Data” tab.
  - To set up a default export content:
    - From the “Definition” drop down menu select “Add new”.



- An “Add New Definitions” window opens up, uncheck “Use Current Settings”
- Name this “BACGene Export”.
- In “File Type” select “Text”.
- In items list, select “Amplification plots” and “Tabular Results”.
- Edit “Tabular Results” by clicking on the blue pen and click “Select All” in the “Column Options” pop-up.
- Press disk icon beside the “Definition” drop down menu to save this export configuration.



5. To export results, follow these instructions

- Go to “Export Data” under the header “Results”.
- Select “BACGene Export” from the Definition drop-down menu.
- Select “Export Data” and save to chosen location.

6. Proceed to “BACGene Evaluation Sheet” for data interpretation.

**FastFinder only**

For evaluation using FastFinder refer to the separate short guide “FastFinder for BACGene kits”.

## 4 BIO-RAD CFX96 TOUCH™ INSTRUCTIONS FOR USE WITH BACGENE SALMONELLA SPP. KIT

The BACGene *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 1.1.

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.

BACGene offers two methods for evaluating the final results. Users can choose between the automated evaluation software FastFinder or the BACGene Evaluation Sheet. Evaluation specific comments are marked in the protocol below in colored boxes:

*Evaluation sheet only*

*FastFinder only*

### 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 us at [kits@eurofins.com](mailto:kits@eurofins.com) for more information.

#### 4.1.1 Before you begin

**Note:** The required protocol and plate layout templates are available via [kits@eurofins.com](mailto:kits@eurofins.com). Furthermore, the CFX Manager™/ CFX Maestro™ 1.1 software can be configured for use with a Laboratory Information Management System “LIMS”. Please contact [kits@eurofins.com](mailto:kits@eurofins.com) 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™ 1.1.
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 software) make sure to import and save the correct plate layout template:

*Evaluation sheet only*

For evaluation with BACGene Evaluation Sheet: “BACGene\_EV\_Salmonella\_spp\_Plate.pltd”

*FastFinder only*

For evaluation with FastFinder: “BACGene\_FF\_Salmonella\_spp\_Plate.pltd”

### 4.1.2 Running a PCR Using a Plate Layout Template

Make sure that the correct protocol and plate layout templates have been imported into the Express Load folder according to section 4.1.1. Alternatively, a LIMS template import can be generated directly from LIMS, for more information please contact us at kits@eurofins.com.

1. Open the CFX Manager™ 3.1 or CFX Maestro™ 1.1 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 BACGene 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 BACGene Evaluation Sheet: “BACGene\_EV\_Salmonella\_spp\_Plate.pltd”

*FastFinder only*

For evaluation with FastFinder: “BACGene\_FF\_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 BACGene *Salmonella* spp. kit and verify the target names for the other assays with the respective assay manual:

BACGene <i>Salmonella</i> spp.	Detection Channel	<i>Evaluation sheet only</i>	<i>FastFinder only</i>
		Target Name	Target Name
<i>Salmonella</i> spp.	FAM™	2014a	S.spp.
IPC	Cy5™	2014i	IPC

**Note:** The target names have to be correct to ensure the correct evaluation using the BACGene Evaluation Sheet or FastFinder software. The correct target names are assigned to the wells in templates provided by us. For more information please contact us via [kits@eurofins.com](mailto:kits@eurofins.com)

- Check sample type definitions:

**Evaluation sheet only**

Sample type definitions for evaluation with BACGene 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 only**

Sample type definitions for evaluation with FastFinder software:

- Name of positive control should start with “C+” (e.g. well A1)
- Name of negative control should start with “C-“ (e.g. well B1)

**Important:** For mixed plates please confirm the parameters using the manuals 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 the BACGene Evaluation Sheet or FastFinder software. The data exports from the Bio-Rad CFX96 Touch™ or Bio-Rad CFX96 Touch™ Deep Well for the BACGene Evaluation Sheet are described below.

The evaluation using FastFinder is described in the separate short guide “FastFinder for BACGene kit”.

*Evaluation sheet only*

### Export of Data for the Evaluation Using the BACGene Evaluation Sheet

1. Open Bio-Rad CFX Manager™ software version 3.1 / CFX Maestro™ software 1.1 and select the saved run. This can be done using one of the following options:
  - a. Double click on the saved run file. This will also automatically open the Bio-Rad CFX Manager™ 3.1 / CFX Maestro™ 1.1 software.
  - b. Files can be dragged and dropped into the open Bio-Rad CFX Manager™ 3.1 / CFX Maestro™ 1.1 software.
  - c. In the open software, go to “File”, then click “Open” and select the run file via the directory.

**Note:** If you are using the cycler via the touch screen without an external computer please contact us at kits@eurofins.com for more information.

2. Once the run file is opened, go to “Settings” tab and ensure the following options are selected:
  - Cq Determination Mode = Single Threshold.
  - Baseline Setting = Baseline Subtracted Curve Fit.
  - Ensure that “Apply Fluorescence Drift Correction” is selected under “Baseline Settings”.
3. To export results, follow these instructions:
  - Go to “Export” tab.
  - Select “All Data Sheets” and export as text files (\*.txt). Choose a location for the files.
  - Got to “Export” and select “Custom Export” choose text format (\*.txt) with the following columns and save in chosen location:
    - Well
    - Fluorophore
    - Target Name
    - Content
    - Sample Name
    - Cq
    - End RFU

**Important:** The order of the exported columns is mandatory.

**Note:** Save all export files in the same folder.

4. Click “Export”
5. In the “Export Complete” pop-up, click “Ok”

6. Proceed to BACGene Evaluation Sheet for data interpretation.

**FastFinder only**

For evaluation using FastFinder refer to the separate short guide “FastFinder for BACGene kits”.

## 5 DATA INTERPRETATION

Data is interpreted using the FastFinder evaluation software or ‘BACGene Evaluation Sheet’ provided.

For FastFinder evaluation please refer to the “FastFinder for BACGene kits” manual.

For an evaluation using the evaluation sheet upload data as described in ‘BACGene Evaluation Sheet’ file.

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

<b><i>Salmonella</i></b>	<b>Internal Positive Control</b>	<b>Final results</b>
Reaction positive	Not relevant	Positive for <i>Salmonella</i> spp.
Reaction negative	Valid	Negative for <i>Salmonella</i> spp.
Reaction negative	Invalid	Questionable*

\* Refer to troubleshooting section (see chapter 7).

## 6 CONFIRMATION OF PRESUMPTIVE POSITIVE RESULTS

In the context of NF VALIDATION and AOAC-PTM certification, all samples identified as positive by the BACGene *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\* and another chromogenic agar followed by latex test (i.e. Oxoid) and biochemical galleries directly on isolated typical colonies without a purification step.

\* Choose a second selective plating medium which is complementary to XLD agar and is based on different diagnostic characteristics to those of XLD agar to facilitate detection of, for instance, lactose positive or H<sub>2</sub>S-negative *Salmonella*. For examples of isolation media, see ISO 6579-1:2017, Annex E.

### Option 3:

Using any other method certified NF VALIDATION or AOAC, respectively, the principle of which must be different from the BACGene *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 BACGene *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

BACGene offers two methods for evaluating the final results. Users can choose between the automated evaluation software FastFinder or the BACGene Evaluation Sheet. Evaluation specific troubleshooting comments are marked below in colored boxes:

*Evaluation sheet only*

*FastFinder only*

### 7.1 Problems upon delivery / during storage and reception

#### **Observation**

***Master mix plate is not frozen upon arrival and without appropriate cooling (dry ice)***

Possible Cause	Solution
Plates defrosted during shipping.	Contact Eurofins GeneScan Technologies Customer Service (kits@eurofins.com or +49-(0)761-5038-200) or your local distributor.

#### **Observation**

***Master mix was thawed.***

Possible Cause	Solution
Freezer problems	Install an external alarm system on your freezers in order to get notice if there are problems.  GeneScan Technologies takes no liability for products stored incorrectly. If you nevertheless would like to test the performance of the product, contact Eurofins GeneScan Technologies Customer Service (kits@eurofins.com or +49-(0)761-5038-200) for guidelines.
Master mix storage was forgotten upon reception and/or not stored at -20°C.	GeneScan Technologies takes no liability for products stored incorrectly. If you nevertheless would like to test the performance of the product, contact Eurofins GeneScan Technologies Customer Service (kits@eurofins.com or +49-(0)761-5038-200) for guidelines.

## 7.2 Problems associated to handling of the plates

### Observation

#### *Frozen master mix 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 Master mix 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.


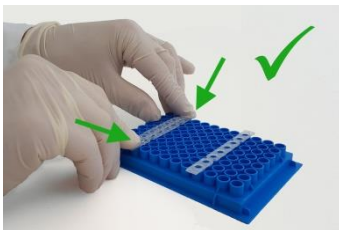
### Observation

#### *Master mix is spilling during removing the seal*

Possible Cause	Solution
Master mix 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.	<p>To facilitate closing the cap strips on PCR strips first softly fix the ends of the cap strip to the reaction tubes, than apply even pressure to tightly close the PCR strips.</p> <div style="display: flex; justify-content: space-around;">   </div>

### 7.3 Incorrect data export and import to BACGene 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 “BACGene Evaluation Sheet” or on the official Microsoft Office website ( <a href="http://office.microsoft.com/">http://office.microsoft.com/</a> ). Then repeat the evaluation.
Data files are not recognized for the import.	<p><u>Agilent AriaMx™:</u>                      Ensure that “Text” is selected as file type in the “Export Data” section.</p> <p><u>Bio-Rad CFX96 Touch™ (Deep Well):</u>                      Ensure that “Text” (as Export Format) and “Tab” (as Column Separator) are selected in the “Custom Export” section.</p>
Relevant data is missing in the export files.	<p>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.</p> <p><u>Agilent AriaMx™:</u>                      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 3.2).</p> <p><u>Bio-Rad CFX96 Touch™ (Deep Well):</u>                      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 4.2).</p>

### 7.4 Incorrect run file upload using FastFinder software

*FastFinder only*

**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 3.1.1 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.	<p style="text-align: right;"><i>Evaluation sheet only</i></p> <p>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.</p> <p>Alternatively: Define the respective Tasks in the original run file and repeat the export and evaluation.</p> <p style="text-align: right;"><i>FastFinder only</i></p> <p>Restart analysis and identify/select correct location of C+ and C-.</p>

**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 Eurofins GeneScan Technologies (kits@eurofins.com or +49-(0)761-5038-200).
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
Master mix plate was freeze-thawed more than 3 times	The master mix 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 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, spin down before use.
C- has not been assigned correctly.	Make sure that the correct well for C- is selected.
Automated threshold calculation failed.	Refer to section 7.6. <span style="float: right;"><i>Evaluation sheet only</i></span>

**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 Eurofins GeneScan Technologies.
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.
Automated threshold calculation failed.	<i>Evaluation sheet only</i> Refer to section 7.6.

*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 7.6.0

**Observation**

***Linear, straight lines instead of amplification curves or amplification curves that lay completely below the threshold***

Possible Cause	Solution
Fluorescence drift was not automatically removed from analyses	Bio-Rad CFX96 Touch™ (Deep Well): Ensure the “Apply Fluorescent Drift Correction” is selected and repeat the export of results. Then continue with the evaluation.
Automated threshold calculation failed.	Refer to section 7.6.

**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).	<p><b>Note:</b> 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.</p> <p>If PCR remains inhibited, repeat lysis of enrichment and repeat PCR.</p> <p>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).</p> <p>If inhibition still persists, review instructions of the respective target specific reference method and the ISO 6887 series for preparation of initial suspensions.</p> <p>Check if the matrix type has been validated for use with the BACGene kit. The method should only be applied to validated matrices.</p>
Automated threshold calculation failed.	<p style="text-align: right;"><i>Evaluation sheet only</i></p> <p>Refer to section 7.6.</p>

## 7.6 Incorrect automated threshold settings in cycler software

**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:

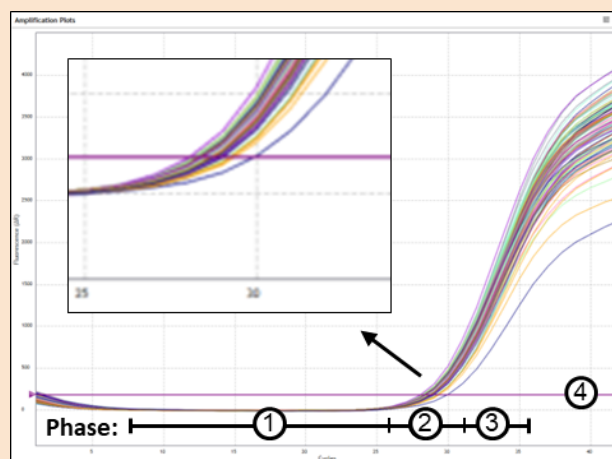


Figure 2: Exemplary amplification plot of a real-time PCR in linear scale (Graph Type “Linear”). 1, background; 2, exponential amplification phase; 3, linear amplification phase; 4, threshold line.

\* not part of the AOAC validation study.





Bio-Rad CFX™ software:

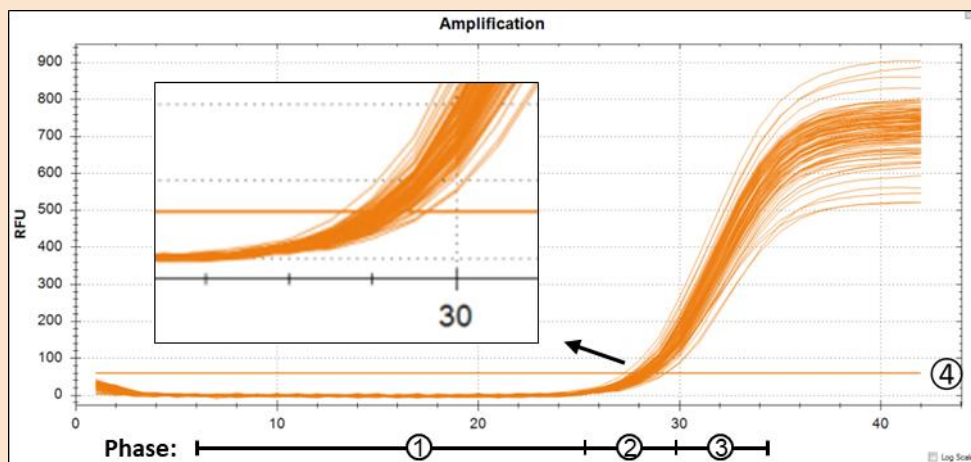


Figure 3: Exemplary amplification plot of a real-time PCR in linear scale (Box "Log Scale" is unchecked). 1, background; 2, exponential amplification phase; 3, linear amplification phase; 4, threshold line.

## 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.	<p><u>General:</u>            Use of <i>PREraser</i> BACGene is recommended for samples in which a high level of free DNA from dead cells is expected. <i>PREraser</i> is applied before lysis of the living cells in order to eliminate signals from free DNA. Refer to the <i>PREraser</i> BACGene manual and relevant certifications for details about the scope and applicability of the method for the matrix in question.</p> <p><u>Environmental samples:</u>            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.</p>

## 8 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

Eurofins GeneScan Technologies GmbH ("GeneScan") warrants the products manufactured by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions for a period equal to or shorter of one year from the date of shipment of the product(s) or the expiration date marked on the product packaging under the storage conditions, recommended in the instructions and/or on the package. GeneScan makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. GeneScan'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 GeneScan promptly of any such defect. GeneScan 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 Eurofins GeneScan GmbH terms and conditions can be obtained on request, and is also provided in our price lists.

## 9 TECHNICAL SUPPORT SERVICE

For technical assistance and more information please contact the Eurofins GeneScan Technical Service or your local distributor.

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