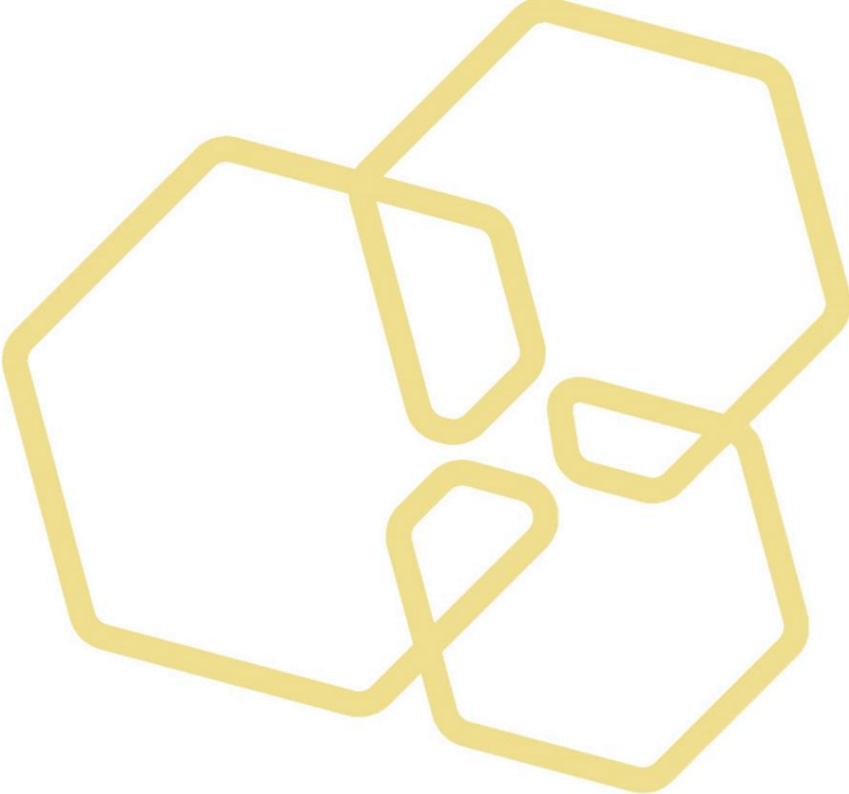




BACGene GO Salmonella

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

HU0060402 BACGene GO Salmonella

For use on Bio-Rad CFX Opus (Deepwell and Standard), Analytik Jena qTOWERiris/GSD version and ThermoFisher QuantStudio™ 5 Fast.



EGS 38/08-03/25
ALTERNATIVE ANALYTICAL
METHODS FOR AGRIBUSINESS
<http://nf-validation.afnor.org/en>



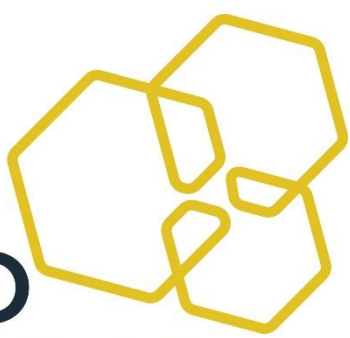

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1 INTRODUCTION

Salmonella is a motile, non-spore forming, Gram-negative, rod-shaped bacterium in the family *Enterobacteriaceae*. The genus *Salmonella* is divided into two species that can cause illness in humans, *Salmonella bongori* and *Salmonella enterica*, the latter being characterized as being the greatest public health concern (Hammack, 2012). Salmonellosis is the second most common foodborne disease within the European Union. According to the European Food Safety Authority (EFSA), between 90,000 to 100,000 thousand cases per year are reported across member states. Of those, around 900 to 1500 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 a consequence of this pathogen. Many foods are prone to contain *Salmonella* species, with eggs and egg products, bakery products, and pork meat being some of the most likely examples to cause outbreaks.

Though there are more than 2555 different serovars of *Salmonella* (Grimont, 2007) described, 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.

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. However, most industries require considerable faster results to be able to release their products on-time, or to initiate any required corrective actions as soon as possible in case of the presence of a pathogen.

Molecular methods based on DNA detection decreased the time to result significantly with a release of negative results in less than 24h. BACGene GO Salmonella, 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 within one hour after the enrichment of a given sample and the extraction of the DNA from the sample by simple mechanical lysis.

Intended Use

The BACGene GO Salmonella detection kit provides materials for the qualitative detection of *Salmonella* spp. DNA from human food, pet food and production environmental samples.

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

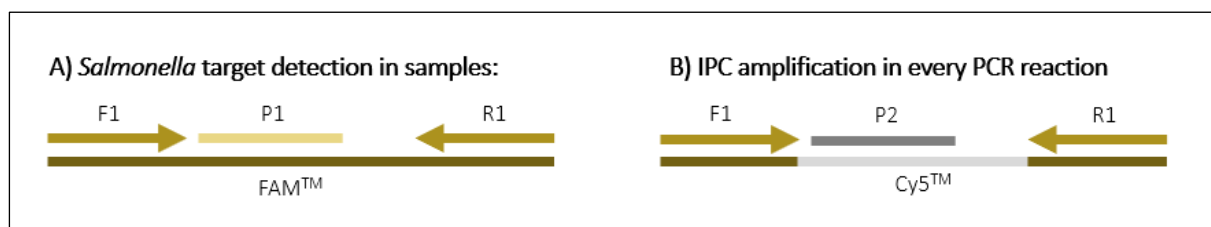
BACGene GO Salmonella kit is certified by AOAC PTM and validated by AFNOR Certification using a manual workflow as an alternative method for *Salmonella* spp. detection in a broad range of foods, Pet food and industrial production environmental samples by comparison to EN ISO 6579-1.

The BACGene GO Salmonella kit is validated for use on Bio-Rad CFX OPUS (Deepwell and Standard), Analytik Jena qTOWERiris/GSD version and ThermoFisher QuantStudio™ 5 (Fast).

1.1 Test Principle

The BACGene GO Salmonella method is a qualitative real-time Polymerase Chain Reaction (PCR) assay for the detection of *Salmonella* spp. in broad range of human foods, pet food, animal feed and environmental samples. DNA amplification and detection take advantage of the nucleotide sequence conservation found in bacterial genomes that allows highly specific and sensitive detection of pathogenic bacteria based on virulence genes. After enrichment, DNA is extracted by a simple mechanical lysis step and rapidly analysed by real-time PCR. By means of specific primers, a nucleotide sequence of *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 via FAM™ fluorescence-labelled probes quenched by non-fluorescent quencher (NFQ). An internal positive control (IPC) is included in the PCR mix. IPC DNA is amplified in parallel and detected using an ATTO647N™ fluorescence-labelled hybridization probe quenched by NFQ. IPC detection indicates proper functioning of the PCR, amplification is measured in the Cy5™ detection channel of the real-time instrument.

Additionally, the IPC in the BACGene GO Salmonella kit is acting as a positive control for correct *Salmonella* amplification, as the same primers are used.



A) *Salmonella* amplification detected in FAM™, B) Amplification of the internal positive control (IPC) in Cy5®, IPC and *Salmonella* amplification share the same primers.

To effectively prevent carry over contamination between PCR assays, the BACGene GO Salmonella kit utilizes Uracil-DNA Glycosylase (UNG). Deoxyuridine triphosphate (dUTP) substitutes for deoxythymidine triphosphate (dTTP) in the PCR mix. Thus, newly synthesized amplicons contain deoxyuridine instead of deoxythymidine. The heat-labile UNG cleaves hydrolytically at the N-glycosylic bond of uracil and deoxyribose and is subsequently inactivated by the initial denaturation step in the PCR. A PCR preceded by this step exclusively amplifies from native thymine-containing DNA (e.g. bacterial DNA), reducing the risk of false positive signals due to carry over contamination from previous amplicons.

To prevent amplification of free-DNA from dead cells in the enrichment sample, the PREraser BACGene protocol is validated to be used for removal of free DNA prior to cell lysis.

1.2 Components of the Kit

Important Notes:

- Storage of the complete kit at 2 – 8 °C, after disassembly of the kit the aluminium bag containing the PCR plate can be stored at 2-25°C. For detailed storage information see the indications in the table below.
- Do not freeze.
- Keep away from light.
- Do not use any components beyond the expiration date, which is printed on the kit box and/or component labels.
- Only remove the required number of tubes and close aluminium bags carefully again to maintain the stability of the remaining components and return to the respective storage conditions.
 - For the PCR plate, make sure that the silica-pouch is included in the aluminium bag over the complete storage time.
- If remaining components are stored correctly (closed aluminium bag including silica-pouch for the PCR plate) according to the conditions stated below, they are stable for 3 months.
- Keep away from light.
- Do not use any components from damaged or incorrectly sealed aluminium bags, refer to CustomerService.BUD@eu.goldstandarddiagnostics.com in such a case.

Cat. no. HU0060402 (96 reactions)

Item	Component	Storage conditions	Expiration date	Expiration date
			Unopened	Opened
1	Complete kit	2 – 8 °C	See kit box and/or label	3 months*
2	Aluminium bag with Ready-to-use lysis plate	2 – 8 °C	See kit box and/or label	3 months*
3	Aluminium bag with lyophilized Ready-to-use PCR plate	2 – 25 °C	See kit box and/or label	3 months*
4	Optical caps bag containing 12 strips	n/a	n/a	n/a

* Correctly closed Aluminium-bag, including Silica-bag for the PCR plate.

1.3 Additional Equipment, Consumables and Reagents Required

1.3.1 For Sampling/Enrichment

- **Swabs/sponges/wipes/cloths** (for environmental samples), premoistened with neutralizing buffer (e. g. Dey-Engley; Lethen broth; HiCap brothy)
- **Buffered Peptone Water (BPW)**, prepared according to EN ISO 6579 and EN ISO 11133 standards.
 - BUFFERED PEPTONE WATER, 10 kg or box (cat. no. 5613902210 or 5613902220)
- **Tween 80** (for food samples with fat >20 % according to ISO 6887 series)
- **Sample preparation** according to EN ISO 6887 for ingredient and specific food
- **Stomacher® or paddle blender**, capable of running between 15 000 and 20 000 rev/mini.
- **Enrichment bags** with side filter:
 - Bag Filter P 400 mL, for 25 g samples (cat. no. HU0060321)
 - Bag Filter P 3500 mL, for 375 g samples (cat. no. HU0060322)
- **"Pipetboy acu 2"**, 0.1 - 100 mL (cat. no. HU0060315) and/or **Serological pipettes** 10 mL (cat. no. HU0060320)

1.3.2 For sample pre-treatment after enrichment and prior to lysis

- **PREraser BACGene**, cat. no. 5123222301 (1x 96) or 5123222311 (10x 96)
- **Peptone-Salt buffer** (for cocoa and cocoa-containing matrices)

1.3.3 For Lysis

- **Lysis rack**, to lock up to 96-lysis tubes for the lysis procedure. Cat. no. HU0060403.
- **Orbital shaker** (radius: 3 mm, speed $\geq$ 2000 rpm)
- **Multichannel** and/or **Single channel pipette**. Cat. no. HU0060312 and / or HU0060307, respectively
- **Filter tips**, DNA-/Nuclease-free pipette tips Cat. no. HU0060317. Alternatively, Filter tips from VWR, Cat. no. 732-3633, or Eppendorf, Cat. no. 30078543 can also be used. (**Important:** The pipette tips must be sufficiently thin to avoid widening the cross-slit in the lysis tube cap during pipetting. Most common 100 μ L tips are too thick. Thus, the references mentioned above must be used. Other references could also be implemented. Please contact technical support for more information)
- **Cluster tube**, 2 mL tube
- **Gloves**, powder-free

1.3.4 For PCR:

- **Multichannel and/or Single channel pipette.** Cat. no. HU0060312 and / or HU0060307, respectively
- **Filter tips,** DNA-/Nuclease-free pipette tips Cat. no. HU0060317. Alternatively, Filter tips from VWR, Cat. no. 732-3633, or Eppendorf, Cat. no. 30078543 can also be used. (**Important:** The pipette tips must be sufficiently thin to avoid widening the cross-slit in the lysis tube cap during pipetting. Most common 100 µL tips are too thick. Thus, the references mentioned above must be used. Other references could also be implemented. Please contact technical support for more information)
- **Real-time PCR Thermocycler:**
 - Bio-Rad CFX Opus 96 Real-Time PCR System (CFX Maestro™ Software 2.3)
 - Bio-Rad CFX Opus Deepwell Real-Time PCR System (CFX Maestro™ Software 2.3)
 - Thermo Fisher Scientific QuantStudio™ 5 (Fast) 0.1 mL (Design & Analysis Software 2.6.0)
 - Analytik Jena qTOWERiris/GSD version (qPCRsoft v5.0.2.1 or later), Cat. no. HU0060911 (equipped with FAM, HEX and Cy5 filters)
 - Analytik Jena qTOWERiris/GSD version (qPCRsoft v5.0.2.1 or later), Cat. no. HU0060912 (equipped with FAM, HEX, CY5 and ROX filters)
- **PURE automated PCR analysis software V1**

1.4 Safety Precautions

- All samples should be handled with caution as they are potentially infectious.
- 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, for the unborn child in case of pregnant women.
- Always wear appropriate Personal Protective Equipment (PPE). The remaining components of the BACGene GO Salmonella kit are not classified according to Regulation (EC) No. 1907/2006 (REACH).
- For more information, please refer to the kits safety data sheet (SDS).

1.5 Working Guidelines

- Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).
- Especially take care to use calibrated pipettes to ensure correct volumes used for lysis and PCR.
- 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-free, nuclease-free and sterile lab ware.
- Wear free-powder gloves.

Change gloves after removing samples from the enrichment, before starting the lysis protocol.

1.5.1 Cleaning Protocol

Assuring workspaces are clean, before, during and after working with BACGene 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.

1.6 Waste Disposal

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

2 HOW TO USE THIS PRODUCT

Important: For every PCR, it is necessary to prepare a negative control reaction.

These are **REQUIRED** for the evaluation of the PCR results.:

- **1 Negative control (C-):** 1 tube containing 20 µL of lysis buffer (leave the lysis well empty, sample-free, during the lysis procedure)

Additionally, enrichment controls are **RECOMMENDED**:

- 1 Positive enrichment control (E+): Enrichment medium with *Salmonella* (e.g. *Salmonella* Tranorooa)
- 1 Negative enrichment control (E-): Enrichment medium *Salmonella*-free

2.1 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.

For homogenisation, use stomacher equipment or hand mixing especially for high sample size testing.

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

AOAC PTM approved protocols (License N° 112402)

Matrix	Enrichment	
	Medium	Incubation
25 g / 25 mL test portion on: • Tabouleh salad (with and without PREraser BACGene^a) • Raw ground beef, 80% lean • Raw milk (with and without PREraser BACGene^a) • Bagged lettuce	1:10 BPW	36 ± 2 °C; 16-24h
25 g / 25 mL test portion on: • Powdered Infant formula with probiotics	1:10 BPW or dsBPW-T ^b	
Surfaces: • Stainless steel: 1 swab (soaked in 1 mL Letheen broth) • Ceramic tiles: 1 sponge (soaked in 10 mL Letheen broth) (with and without PREraser BACGene^a)	10 mL BPW (swab) / 100 mL BPW (sponge)	
Up to 375 g Chocolates and Cocoa-based products (Cocoa powder, raw Ingredients, etc.) ^c	Option 1: Sample in 1:10 pre-warmed BPW	36 ± 2 °C; 20-28h
	Option 2: Sample in 1:10 pre-warmed UHT milk or NFDM according to ISO 6887-4 ^c	36 ± 2 °C; 22-30h
Up to 375 g Pet Food	Sample in 1:6 pre-warmed BPW (ie: 375 g in 1875 mL pre-warmed BPW)	36 ± 2 °C; 20-28h

^aThe optional pre-treatment with PREraser BACGene was validated and certified on three representative matrixes, but is applicable for the whole scope after the validation.

^bNote: Double strength BPW with Tween 80

^cNote: For cocoa and cocoa-containing matrices a dilution in peptone-salt (1 mL primary enrichment + 9 mL peptone-salt buffer) has to be performed after enrichment and prior to lysis

2.1.1 Matrices Certified NF VALIDATION by AFNOR Certification (Certificate number EGS: 38/08-03/25) according to ISO 16140-2

The BACGene GO Salmonella kit is certified by AFNOR Certification as an alternative method for Salmonella spp. detection according to EN ISO 16140 and by comparison to the international standard ISO 6579-1 and its amendment A1 for the scope listed in the table below.

The EGS 38/08-03/25 certificate can be obtained from CustomerService.BUD@eu.goldstandarddiagnostics.com or from AFNOR Certification. The date of end of validity for the NF VALIDATION certification is indicated on the certificate available on the NF VALIDATION website <http://nf-validation.afnor.org/en>.

Matrix	Enrichment	
	Medium	Incubation
25 g All human food products (except cocoa, spices and dehydrated herbs)	1:10 BPW	36 ± 2 °C; 16-24h
25g Dusts, 25 mL process water		
25 g cocoa, spices and dehydrated herbs	Follow ISO 6887-4 sample preparation instructions	
1 Swab ^a	10 mL BPW	
1 Sponge ^a	100 mL BPW	
Up to 375 g Chocolates and Cocoa-based products (Cocoa powder, raw Ingredients, etc.) ^c	Option 1: Sample in 1:10 pre-warmed BPW	36 ± 2 °C; 20-28h
	Option 2: Sample in 1:10 pre-warmed UHT milk or NFDM according to ISO 6887-4 ^c	36 ± 2 °C; 22-30h
Up to 375 g Pet Food	Sample in 1:6 pre-warmed BPW (i.e.: 375 g in 1875 mL pre-warmed BPW)	36 ± 2 °C; 20-28h

^a For Sampling before cleaning process, use BPW only for enrichment. For Sampling after cleaning process, use BPW with 10% of neutralizing agent

^b For example, 375 g of sample to 3 375 mL of media. Addition of Brilliant Green (0.018 g/L) might be required for product with high background microflora

^cFor cocoa and cocoa-containing matrices a dilution in peptone-salt (1 mL primary enrichment + 9 mL peptone-salt buffer) has to be performed after enrichment and prior to lysis

Note: *PREraser BACGene* can be optionally integrated into the workflow for removal of free DNA for the whole certified matrix scope.

2.2 Lysis

Special precautions:

- Label lysis tubes to ensure correct orientation when pipetting samples.
- **Filter tips**, DNA-/Nuclease-free pipette tips Cat. no. HU0060317. Alternatively, Filter tips from VWR, Cat. no. 732-3633, or Eppendorf, Cat. no. 30078543 can also be used. (**Important:** The pipette tips must be sufficiently thin to avoid widening the cross-slit in the lysis tube cap during pipetting. Most common 100 µL tips are too thick. Thus, the references mentioned above must be used. Other references could also be implemented. Please contact technical support for more information)
- For Chocolates and Cocoa-based products, Dilute the enrichment 1:10 before starting the lysis step (For example: 100 µl enrichment in 900 µl of sterile BPW or Peptone Salt)
- To prepare the lysate, proceed with lysis step immediately or store the retained enrichment at 2-8°C for 72 hours. Do not exceed 72 hours of total storage time.

Lysis steps:

1. **Transfer 20 µL of enrichment into appropriate lysis tube.** Proceed to the next lysis sample/strip until all samples have been included.
2. **Place lysis tube/strips/plate onto the orbital shaker at 2000 rpm for 10 min.**

2.3 PCR

Special Precautions:

- Separate the different procedures spatially. Ideally, use separate rooms for sample preparation and PCR setup, or at least dedicated areas, equipment, and consumables for each procedure.
- Use DNA/Nuclease-free filter tips.
- Wear disposable powder-free gloves.
- Store BACGene GO Salmonella kit components for PCR and lysis only in dedicated areas, and apart from enrichment preparation areas and sample storage.
- Preferably use 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, 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).

PCR steps:

1. **Open PCR plate** by peeling of the foil.
Note: Only open one PCR strip at a time to avoid cross-contamination.
2. **Transfer 20 µL of each lysate** by piercing the lysis tube/s (using single channel or multichannel pipette) to the corresponding PCR strip.
Note: Make sure that the lysate is taken without removing any bead. Enhance the piercing by tilting the pipette when piercing in and out. Ensure the lysate is pipetted-down directly on top of the lyophilized PCR mix.
3. **Close the PCR strip with an optical cap** strip with clean, gloved hands.
4. **Homogenize** by briefly vortexing or manually pipetting up/down. Briefly spin down, if needed.
5. **Transfer the PCR plate to the real-time instrument** and **start the run** (refer to section 3).

3 ANALYTIK JENA qTOWERiris/GSD VERSION: INSTRUCTIONS FOR USE WITH BACGENE GO SALMONELLA KIT

The BACGene GO Salmonella kit is validated for the use on:

RT PCR device	Software Version	Dye	Detection channel	Ramping/Instrument Setting
Analytik Jena qTOWERiris/GSD version	qPCRsoft v5.0.2.1 or later	1) FAM 2) Atto647N	1) FAM (Salmonella) Gain settings 4 2) Cy5 (IPC) Gain settings 5	Heating/cooling rate set to 2.5 °C/s Activate "STC" option

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 equipment.

3.1 Running a PCR

The following instructions outline how to set up a PCR run on the Analytik Jena qTOWERiris/GSD version using an external computer.

Note: If you are using the cycler via the touch screen without an external computer, please contact CustomerService.BUD@eu.goldstandarddiagnostics.com for more information.

3.1.1 Kit configuration

6. Ensure the “Thermal Profile” and “Dyes” programmed on the run are according to the tables above (dyes/detection channels, instrument settings) and below (PCR profile) as per the kit configuration.
7. A file template (.rtsx) containing all the PCR parameters described below is available from sales support, please see section 3.1.2.

Step	Stage	T in °C	Time in sec	Data collection	Cycles
1	UNG Digestion	50	120	No	1x
2	Initial enzyme activation	95	120	No	1x
3	Amplification:				
	3a) Denaturation	95	15	No	41x repetitions (42x cycles)
	3b) Annealing and Extension	60	20	yes	

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

3.1.2 Data import

Note: The necessary run template (.rtsx) file is available from sales support.

1. Turn on Analytik Jena qTOWERiris/GSD version Real-Time PCR System.
2. Open the qPCRsoft v5.0.2.1. or later
3. Select (one of) the instrument(s) connected to the computer.
4. When setting up a computer/instrument combination for the first time:
 - 4.1. Go to “Extras” in the top bar, select “Options” and go to the “General” section.
 - 4.2. Define the default “Auto save folder (Project)” folder you want to save the raw data (instrument run files).
 - 4.3. In the “Save projects” section, activate “Automatically”, define desired file name options and select “*.rtpx” as file format.
 - 4.4. Activate “Save backup file” and both automatic “.csv” data export checkboxes.
 - 4.5. Define a folder, where the automated exports are saved.
 - 4.6. Confirm your settings using the “OK” button.
 - 4.7. Steps 7.1 to 7.5 only need to be done during the initial set up (for more detailed information, please refer to instrument manual).
5. Go to “File” in the top bar and select “Open Template”.
6. Navigate to the project templates and select: **BACGene_GO_Salmonella_V1.rtsx**
7. Plate set up:
 - 7.1. Go to the “Samples” section.

- 7.2. Assign “Unknown” as sample type for all wells of the plate.
 - 7.3. Name the negative control as “C-“. Analytical samples can be named according to your regular sample nomenclature or as e.g. “sample”. Please ensure that for every well containing an analytical sample information is included in the “sample name” field.
 - 7.4. For FAM™ and Cy5®, “2035a” and “2035i”, respectively, need to be assigned in the “Gene” field.
 - 7.5. All respective information is included in the templates provided (CustomerService.BUD@eu.goldstandarddiagnostics.com). In case the position of your negative control deviates from the default layout (“C-“ located in well H12), please adjust the layout accordingly.
8. Open the cover of the instrument, load your plate / strips and close the cover.
 9. Click on the “Start” button in the instrument software.

3.1.3 Data export

1. After the PCR run is finished, a pop-up window appears in the instrument software.
2. Confirm by pressing the “OK” button.
3. Data exports files are automatically generated and saved at the default location

4 QUANTSTUDIO™ 5 (Fast): INSTRUCTIONS FOR USE WITH BACGENE GO SALMONELLA KIT

The BACGene GO Salmonella kit is validated for the use on:

Real time PCR device	Software Version	Dye	Detection channel	Ramping Rate
Thermo Fisher Scientific QuantStudio™ 5 (Fast), 0.1 mL	Design and Analysis Cyclor Software 2.6.0	1) FAM 2) Atto647N	1) FAM (Salmonella) 2) Cy5 (IPC)	Ramp rate set to 2 °C/s

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 Equipment.

4.1 Running a PCR

The following instructions outline how to set up a PCR run on the Thermo Fisher Scientific QuantStudio™ 5 (Fast), 0.1 mL using an external computer.

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

Note: The Thermo Fisher Scientific QuantStudio™ 5 software is able to interface with a Laboratory Information Management System “LIMS”. Please contact your local sales support for more information.

4.1.1 Kit configuration

1. Ensure the “Thermal Profile” and “Dyes” programmed on the run are according to the tables above (dyes/detection channels, instrument settings) and below (PCR profile) as per the kit configuration.
2. A file template (.edt) containing all the PCR parameters described below is available from GSD sales support, please see section 4.1.2. Please contact CustomerService.BUD@eu.goldstandarddiagnostics.com.

Step	Stage	T in °C	Time in sec	Data collection	Cyclers
1	UNG Digestion	50	120	No	1x
2	Initial enzyme activation	95	120	No	1x
3	Amplification:				
	3a) Denaturation	95	15	No	42x
	3b) Annealing and Extension	60	30	yes	

4.1.2 Data import

Note: The necessary run template (.edt) file is available from sales support. Alternatively, a run template can be generated from LIMS, please contact us for more information.

1. Turn on Thermo Fisher Scientific QuantStudio™ 5 cycler.
2. Open the Thermo Fisher Scientific QuantStudio™ 5 software (Open Design and Analysis Cycler Software 2.6.0), per default “Quantification View” is shown.
3. Import the Run file Template with an USB Stick.
 - 3.1. On the touch Screen select “Load Experiment”.
 - 3.2. Select “USB” → “Manage files”.
 - 3.3. Browse to the folder with the **Template File Name** and select it:
BACGene_GO_Salmonella_Fast_V1.edt
 - 3.4. Select the template and select “Copy Files”.
 - 3.5. Go back to “Load Experiment” screen by clicking in the upper left corner.
 - 3.6. Select “My Instrument”.
 - 3.7. Click “Manage Files” → “Public” and select “Paste Files”.
4. Touch “My Instrument” → “Public”.
5. Select the **Template file Name**.
6. Change the Run **Template File Name** according to your laboratory specifications.
7. Open the instrument drawer by selecting the icon in the upper right corner.
8. Insert your plate and close the drawer by touching the icon again.

Note: Use the tray “MicroAmp™ 96-Well Tray for VeriFlex™ Blocks” (cat. no. 4379983) for running the BACGene GO Salmonella PCR on the Thermo Fisher Scientific QuantStudio™ 5 instrument.

9. Select “Start run”.

4.1.3 Data export

1. Open **run file** with cycler Software (Design and Analysis Cycler Software 2.6.0) and per default “Quantification View Quality Check” tab is shown.
2. Go to “Plate setup” and click on “+” icon which is at the top right corner in the “Samples” and “Biogroup” section.
 - 2.1. Mark whole plate and update value as “Sample” under “Name” column and “Unknown” under “Type” column. Activate the checkbox next to the sample name and the respective information is assigned to all wells of the plate.
 - 2.2. Define the position of the negative control: Select only the well for the negative control by left click in the plate layout. Click on “+” icon in the “Samples” and “Biogroup” section, enter “C-” in the “Name” column and select “Negative control” from the “Type” column. Click on checkbox on the lefthand side of “C-” and the respective information is assigned to the well containing the negative control.
 - 2.3. Under the “Target” tab (below Sample and Biogroup) ensure target values are ticked and Target Name is updated according to the kit configurations (4.1.1).
 - 2.4. Click on the “Analyze” button, warning icon present on the “Analyze” button should be changed to green tick mark ensuring all the modifications are analyzed.
3. Once plate set up is done, go to “Quality Check” tab in “DATA ANALYSIS” screen.
 - 3.1. Click on “Settings” icon, under “General” section “Y value” should be changed to “Rn”.
 - 3.2. Select all the samples from Plate setup which is below graph section.
 - 3.3. Click on three dots on the top right-hand side and select “Mark Cq as undetermined”.
 - 3.4. Click on “Analyze” button, the warning icon should change to green tick mark.
4. Once the experiment has been analysed and a green tick mark is visible on top of the “Analyze” button, the experiment is ready to be exported.
 - 4.1. To export, click on the “Actions” button. A dropdown appears.
 - 4.2. Select “Export”. A pop-up appears.
 - 4.3. Make the needed changes in the name and destination path. Select the file format as .xlsx and the export setting as Default Export Setting.
 - 4.4. Select the checkbox present at the bottom of the pop-up to export all the tables in a single file.
 - 4.5. Click on the “Export” button: On successful export, a message will be displayed on the top right-corner of the screen.

5 CFX OPUS: INSTRUCTIONS FOR USE WITH BACGENE GO SALMONELLA KIT

The BACGene GO Salmonella kit is validated for the use on:

RT PCR device	Software Version	Dye	Detection channel	Ramping Threshold
Bio-Rad CFX Opus Deepwell Real-Time PCR System	CFX Maestro 2.3	1) FAM	1) FAM (Salmonella)	Ramp rate set to 2.5 °C/s
Bio-Rad CFX Opus Standard Real-Time PCR System		2) Atto647N	2) Cy5 (IPC)	

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 equipment.

5.1 Running a PCR

The following instructions outline how to set up a PCR run on the Bio-Rad CFX Opus 96 Real-Time PCR System (CFX Maestro™ Software 2.3) and Bio-Rad CFX Opus Deepwell Real-Time PCR System (CFX Maestro™ Software 2.3) using an external computer.

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

Note: The Bio-Rad CFX Opus software is able to interface with a Laboratory Information Management System “LIMS”. Please contact your local sales support for more information.

5.1.1 Kit configuration

1. Ensure the “Thermal Profile” and “Dyes” programmed on the run are according to the tables above (dyes/detection channels, instrument settings) and below (PCR profile) as per the kit configuration.
2. Protocol (.prcl) and plate layout (.pltd) template files containing all the PCR parameters described below is available from sales support, please see section 5.1.2.

Step	Stage	T in °C	Time in sec	Data collection	Cyclers
1	UNG Digestion	50	120	No	1x
2	Initial enzyme activation	95	120	No	1x
3	Amplification:	3a) Denaturation	95	No	42x
		3b) Annealing and Extension	60	yes	

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

5.1.2 Data import

Note: The necessary run template (.pltd) file is available from sales support. Alternatively, a run template can be generated from LIMS, please contact us for more information.

1. Turn on Bio-Rad CFX Opus 96 Real-Time PCR System or Bio-Rad CFX Opus Deepwell Real-Time PCR System.
2. Open the Bio-Rad CFX Maestro™ 2.3.
3. Choose “Run setup”.
4. Select instrument CFX Opus 96 or CFX Opus Deepwell.
5. Select run type “User-defined”.
6. In the protocol tab choose “Select existing...”.
 - 6.1. Navigate to the protocol template and select: **BACGene_GO_V1.prcl**
 - 6.2. Select “Next”.
7. In the plate tab choose “Select existing...”.
 - 7.1. Navigate to the plate template and select: **BACGene_GO_Salmonella_V1. pltd**
 - 7.2. Select “Next”.
8. Optional for naming the samples:
 - 8.1. Select “Edit Selected...”.
 - 8.2. Select “Spreadsheet View/Importer” from the panel or from “Editing tools” menu.
 - 8.3. Include the sample names into the list.
 - 8.4. Select “OK”.
 - 8.5. Select “OK” again to leave the plate view.
 - 8.6. Select “Next” to get to the “Start run”.
9. Click “Open Lid”, load the plate into the cycler and then press “Close Lid”.
10. Click “Start Run”.
11. In the directory, choose the location to save the run file and then click “Save”.

5.1.3 Data export

1. Open run file with cycler Software and the “Data Analysis” window is opened - per default “Quantification View Quality Check” tab is shown.
2. If necessary, go to “View/Edit Plate” in the “Plate Setup” dropdown menu and ensure that following parameters are set-up:
 - 2.1. All fluorophores used in the assay are selected and respective target names are assigned according to the kit configuration (see 5.1.1).
 - 2.2. Sample type “Unknow” is selected for the whole plate.
 - 2.3. The negative control is named “C-”, while analytical samples can be named according to your regular sample nomenclature or as e.g. “sample”. Please ensure that for every well containing an analytical sample, information is included in the “sample name” field.

- 2.4. Leave the “Plate setup” and confirm application of settings.
3. In the “Data Analysis” window go to the “Settings” drop down menu:
 - 3.1. Set “Baseline settings” to “No Baseline Subtraction”.
4. In the “Data Analysis” window go to the “Export” drop down menu:
 - 4.1. Select: “Export All Data Sheets” and choose “.csv” file format. Note: Decimal settings need to be “.” separator.
 - 4.2. Select: “EXPORT RDML” and choose “RDML v1.1” as file format.

6 DATA ANALYSIS USING PURE

6.1 Data import to PURE

1. Open the PURE web application and sign in with your credentials.
 2. Press “Get Started” button and select your laboratory.
 3. Select “Home” screen to see previous analysis from your laboratory or to start a new analysis.
 4. To analyse a new PCR run, either drag-and-drop the data files exported from the cycler software on the “Drop PCR-Data Here” section or click on “Select Required File(s)” and navigate to the required export file(s).
- Depending on the real-time instrument used, following export files are required:
- 4.1. ANALYTIK JENA qTOWERiris/GSD VERSION: Either „.ajq” file or all single “.csv” files
 - 4.2. QUANTSTUDIO™ 5 (Fast): “.xlsx” file
 - 4.3. CFX OPUS: All “.csv” files and the “.rdml” file

6.2 Data Evaluation

For evaluation using PURE refer to the separate short guide “PURE”, inside the software.

6.3 Data interpretation

Data is interpreted using the PURE evaluation software. Interpretation of final sample results is summarised in the following table:

<i>Salmonella</i>	Internal Positive Control (IPC)	Results prompted on the software
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 8).

7 CONFIRMATION OF PRESUMPTIVE POSITIVE RESULTS

In the context of NF VALIDATION and AOAC-PTM certification, all samples identified as positive by the BACGene GO Salmonella kit must be confirmed as described below. Confirmation must be performed within 72 hours after the incubation period using the enrichment broth stored at 2-8°C.

- Streak 10 µL of the enrichment broth directly onto XLD, Chromogenic Salmonella Agar (Biolife, CAT# 545359) or IRIS Salmonella Agar (BIOKAR, CAT# BM16008). Incubate agar plates following manufacturer instructions.
- Confirm well-isolated typical *Salmonella* colonies with Latex test (Microgen Salmonella F42) or Biochemical galleries (Microgen GN A ID, MID64) following manufacturer instructions.

Colonies can also be confirmed using the conventional tests described in the methods standardized by CEN or ISO 6579-1:2017/Amd 1:2020 from colonies (including the purification step).

In the event of discordant results (positive with the alternative method, not confirmed by one of the options described above), the laboratory must take the necessary steps to ensure that the results obtained are accurate.

If no typical colonies observed on the agar plates mentioned above, It is recommended, for example, to perform the following procedure:

- Transfer 100 µL from the enrichment broth in 9.9 mL RVS and incubate 24 ± 3 h at $41.5 \text{ °C} \pm 1 \text{ °C}$.
- Streak 10 µL of the incubated RVS broth onto XLD, Chromogenic Salmonella Agar (Biolife, CAT# 545359) or IRIS Salmonella Agar (BIOKAR, CAT# BM16008). Incubate agar plates following manufacturer instructions.
- Confirm well-isolated typical *Salmonella* colonies with Latex test (Microgen Salmonella F42) or Biochemical galleries (Microgen GN A ID, MID64) following manufacturer instructions.

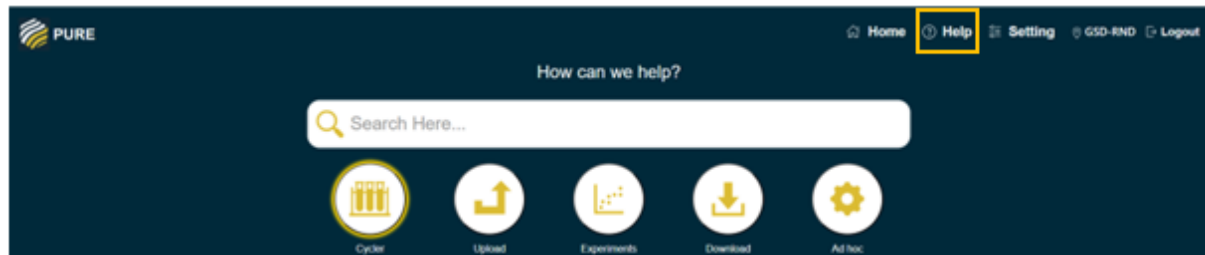
Colonies can also be confirmed using the conventional tests described in the methods standardized by CEN or ISO 6579-1:2017/Amd 1:2020 from colonies (including the purification step).

In the context of NF VALIDATION, positive result obtained by BACGene GO Salmonella PCR can also be confirmed using another method validated according to the standard ISO 16140-2 and certified by NF VALIDATION, which is based on a different principle. The protocol of the second validated method used for confirmation must be respected in its entirety. All steps before the detection step used as a starting point for confirmation, must be common to both methods. Thus, the BACGene GO Salmonella kit and the second validated method must have common enrichment conditions (i.e. Media, Temp, Incubation time).

8 TROUBLESHOOTING

8.1 Data evaluation & interpretation of the results

Please refer to the PURE “Help” section inside the software for all PURE-related questions.



8.1.1 Interpretation of the result as Questionable

Possible Cause	Solution
Inhibition of PCR or inconclusive results (refer to “Data interpretation”)	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. Also, the usage of stomacher bags with filter are recommended for particle rich matrices. If PCR remains inhibited, dilute the lysate with DNA-/Nuclease free water (one-part sample plus nine parts water). If PCR remains inhibited, repeat lysis of enrichment, and repeat PCR. Check if the matrix type has been validated for use with the BACGene GO kit.

8.1.2 Interpretation of the result not possible due to errors in negative control (C-)

Possible Cause	Solution
C- shows a positive Salmonella reaction	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 GSD support.
C- has not been assigned correctly.	Make sure that the correct well for C- is selected. No negative control set. Evaluation is not possible, please go to ‘results – tab view’ and define the position of the negative control (C-) in the column ‘Task’.

8.1.3 Cultural confirmation

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 before transferring an aliquot 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. Alternatively, pipette 10 µL of the enrichment broth, transfer to the agar plate then streak.
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).
Free DNA from dead target organism cell in the sample produces a positive PCR result.	General: 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 to eliminate signals from free DNA. Refer to the <i>PREraser</i> BACGene 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.

9 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.

The 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.

10 LIMITATIONS FOR USE

The BACGene GO Salmonella kit is designed to detect DNA from target organisms as specified when grown to detectable levels during enrichment.

The BACGene GO Salmonella kit has been tested broadly against many, but not all strains. Following 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 BACGene GO Salmonella is capable to detect every single strain or mutants of the genus.

The BACGene GO Salmonella kit has been tested using a vast variety of matrices following ISO 16140-2 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 BACGene results. It is recommended to refer to ISO 16140-3 for method verification. The performance, reliability and results validity of BACGene GO Salmonella PCR assay are dependent on the end-user strict respect of handling steps, verification and implementation of necessary controls.

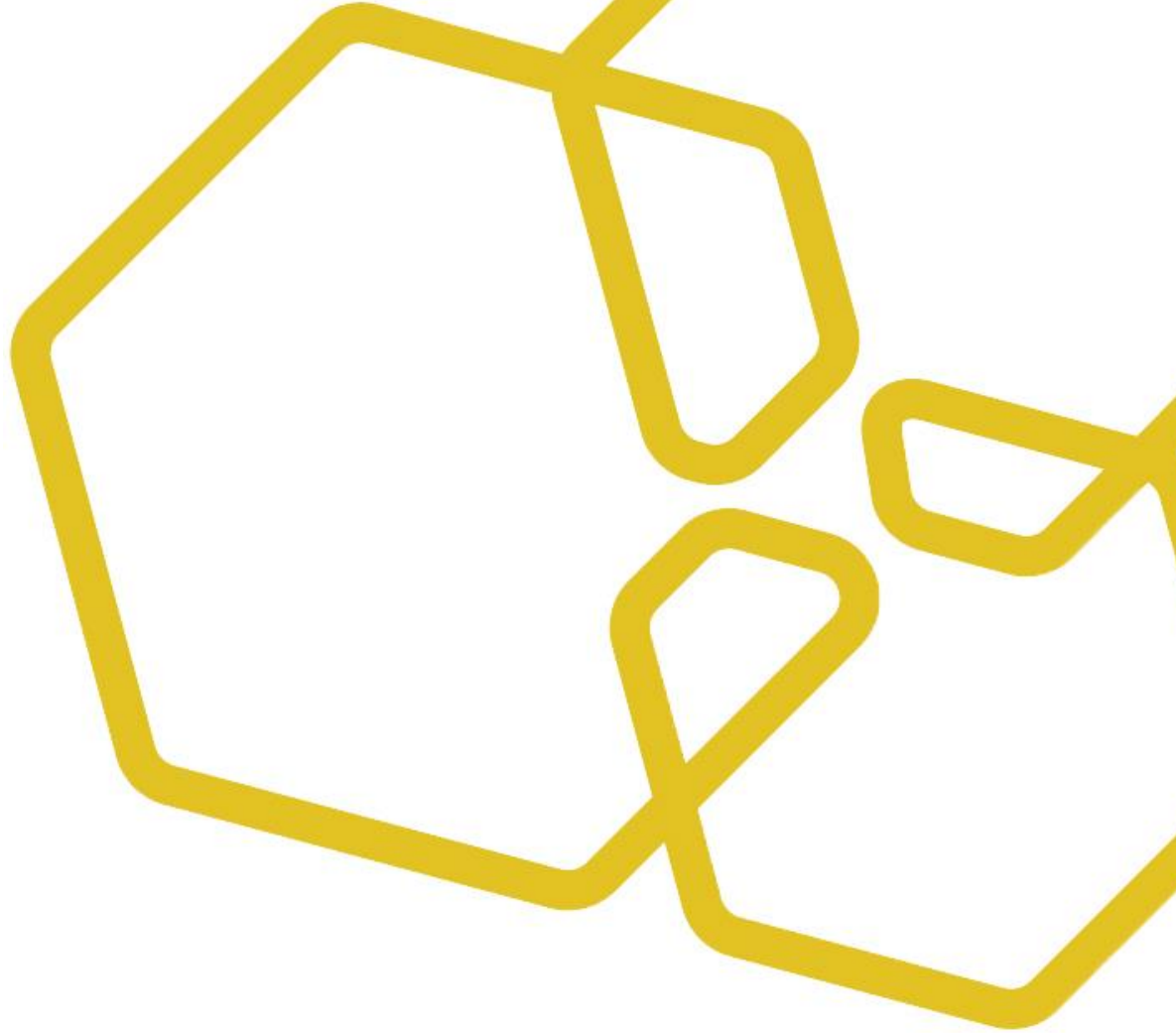
11 IMPORTANT NOTES

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12 VERSION CONTROL

Version	Date	Changes
V3	31.05.2026	• Addition of Matrices extensions (Cocoa and Pet Food) • Adjustment of Confirmation steps description • Correction of Limitations for Use and PURE description • Addition of pipettes and tip information and references • Addition of new later qTOWERsoft versions and new SKU for 4plex instrument • Various format adjustments
V2.1	22.10.2025	• Adjustment of storage conditions • Addition of the matrix Dusts, process water (25 g)
V2	06.03.2025	• Addition of paragraph explaining the NF VALIDATION above matrix table • Addition of final AFNOR certification number in matrix table • Clarification of Confirmation Option 3
V1.8	05.02.2025	• Update of AOAC review comments • Adaptation enrichment temperature from 37+/-1°C to 36+/-2°C • Version control section added • PURE evaluation included as separate section and removed from sections 3, 4 and 5 • Elaboration of further details regarding the design of the internal positive control (Chapter 1.2)



TECHNICAL SUPPORT SERVICE

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

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