foodproof® *Salmonella* plus *Cronobacter* Detection LyoKit

Revision C, May 2025

PCR kit for the qualitative detection of *Salmonella* spp. and *Cronobacter* spp. DNA using real-time PCR instruments.

Product No. KIT230131 (LP)

Product No. KIT230132 (RP)

Product No. KIT230133 (DP)

Kit for 96 reactions (lyophilized) for a maximum of 94 samples

Store the kit at 2 to 8 °C

QUA 18/12-12/24 Detection method for *Salmonella* spp.
QUA 18/13-12/24 Detection method for *Cronobacter* spp.
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

For food testing purposes.
FOR IN VITRO USE ONLY

1. What This Product Does

1.1 Number of Tests

This foodproof® kit is designed for 96 reactions with a final reaction volume of 25 µL each for foodproof Lysis Kits and 30 µL for BAX® Prep Lysis Kit. Up to 94 samples (single sample preparation) plus positive and negative control reactions can be analyzed per run.

1.2 Storage and Stability

- Store the kit at 2 to 8 °C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following Kit Components table.

1.3 Kit Components

Component	Label	Contents / Function / Storage
foodproof *Salmonella* plus *Cronobacter* Detection LyoKit Microplate prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230131 with white low profile tubes (LP)* • KIT230132 with clear regular profile tubes (RP)* • KIT230133 with clear low profile tubes (DP)*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for *Salmonella, Cronobacter* and the Internal Control (IC) as well as Taq DNA Polymerase and Uracil DNA Glycosylase (UNG, heat labile) for prevention of carryover contamination. • For amplification and detection of *Salmonella*- and *Cronobacter*-specific sequences. • Store at 2 to 8 °C in the aluminum bag (sealed and containing silica gel pads). • **Protect from light and moisture!**
Control Template	Vial 2 (purple cap)	• 1 x 250 µL • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.
H_2O, PCR-grade	Vial 3 (colorless cap)	• 2 x 1 mL • Nuclease-free, PCR-grade H_2O. • For use as a PCR negative control.
Cap Strips	Plastic bag containing 8-cap strips	• 12 x 8-cap strip • For use in real-time PCR after addition of samples.

*Tube profile and instrument compatibility chart is available online.

1.4 Additional Required Equipment and Reagents

- Real-time PCR cycler suitable for detection of FAM-, HEX- and ROX-labeled probes as well as for using low or regular profile strip tubes. If the strip tubes do not fit your instrument, the samples should be transferred to appropriate PCR tubes after resuspension of the lyophilized PCR mix.

- DNA extraction kit options (choose one):
  - foodproof StarPrep Three Kit (Product No. KIT230187)
  - foodproof StarPrep Three 8-Strip Kit (Product No. KIT230188)
  - foodproof Magnetic Preparation Kit I (Product No. KIT230180)
  - BAX Prep Lysis Kit (Product No. KIT2047)

  Important note: For detailed information on the lysis procedures, please refer to the corresponding product instructions.

  DNA extraction methods approved by AFNOR Certification: foodproof StarPrep Three Kit, foodproof StarPrep Three 8-Strip Kit and BAX Prep Lysis Kit.

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Optional vortex centrifuge (choose one):
  - Multispin MSC-6000 for PCR-strips with the SR-32, Rotor for MSC-3000/6000
  - CVP-2 for PCR-plates

1.5 Applicability Statement

The foodproof *Salmonella* plus *Cronobacter* Detection LyoKit is intended for the rapid and simultaneous detection of *Salmonella* and *Cronobacter* isolated by appropriate DNA extraction methods from enrichment cultures of all relevant kinds of food and production environmental samples that are potentially contaminated with *Salmonella* spp. and/or *Cronobacter* spp.

This kit must not be used in diagnostic procedures.

This kit has been developed for real-time PCR instruments with detection channels for FAM, HEX (or VIC/Yakima Yellow) and ROX (or Texas Red). The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480, LightCycler 96 (Roche Diagnostics), Mx3005P® qPCR System (Agilent Stratagene), AriaMx® System (Agilent Technologies), ABI 7500 fast (Thermo Fisher), CFX96™ System (Bio-Rad) and PikoReal® 24 (Thermo Fisher).

The performance of the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit in combination with foodproof StarPrep Three (single tube and 8-strip format) and BAX Prep DNA extraction procedures has been approved by AFNOR Certification. Within this NF VALIDATION™ study according to ISO 16140-2 protocol the following food categories have been tested: infant formula with and without probiotics, ingredients and production environmental samples (excluding primary production stage samples). A LightCycler® 480 instrument (Roche Diagnostics) and a BAX® System Q7 instrument (Hygiena®) have been used for PCR analysis.

For further information on the matrices, enrichment protocols and DNA extraction procedures tested, please refer to Appendix 1 at the end of the manual.

Note: A color compensation (Color Compensation Set 5; Product No. KIT230011) is necessary and will be supplied by Hygiena Diagnostics GmbH for users of the LC 480 Systems I and II. Please contact us for further information.

Note: For users of the Agilent AriaMx, it is recommended to use transparent tubes.

2. How to Use This Product

2.1 Before You Begin

2.1.1 Precaution

Detection of *Salmonella* and/or *Cronobacter* DNA using the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit requires DNA amplification by PCR. The kit provides all reagents required for the PCR. However, to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard) and follow the instructions below to avoid nuclease-, carryover-, or cross-contamination:

- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettors, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- To avoid carryover contamination, transfer the required solutions for one experiment into a fresh tube, rather than directly pipetting from stock solutions.
- Physically separate the workplaces for DNA preparation, PCR setup and PCR to minimize the risk of carryover contamination. Use a PCR hood for all pipetting steps.

Keep the foodproof *Salmonella* plus *Cronobacter* Detection lyophilized PCR mix away from light and moisture.

2.1.2 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For preparation of genomic DNA from various sample enrichments, refer to the corresponding product package inserts of a suitable sample preparation kit (see 1.4 Additional Required Equipment and Reagents).

2.1.3 DNA Extraction

Hygiena provides sample preparation kits suitable for all kinds of food and environmental samples (see 1.4 Additional Required Equipment and Reagents).

2.1.4 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [foodproof *Salmonella* plus *Cronobacter* Detection Control Template (vial 2, purple cap)] or with a positive sample preparation control.

2.1.5 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H_2O, PCR-grade (vial 3, colorless cap). Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.1.6 Confirmation

Positive samples can be confirmed with the reference culture methods according to EN ISO 6579-1 for *Salmonella* detection and EN ISO 22964 for *Cronobacter* detection, or any appropriate methods according to the ISO 16140-6 or ISO 7218 standards.

2.2 Procedure

2.2.1 Program Setup

The following procedure is optimized for a real-time PCR instrument with detection channels for FAM (*Salmonella*), HEX (*Cronobacter*) and ROX (Internal Control). Program the PCR instrument before preparing the samples. Use the following real-time PCR protocol for the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit. For details on programing the experimental protocol, see the Instrument Operator's Manual for your real-time PCR cycler.

Note: When using the BAX System Q7 instrument, no program setup needs to be done. Be sure to select 'foodproof – RT Sal spp + Cronobacter' as the target from the drop-down menu.

Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes
Amplification	**50** cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds

* Fluorescence detection occurs in Step 2

Notes:

- For some real-time PCR instruments, the type of the probe quencher as well as the use of a passive reference dye must be specified. The foodproof *Salmonella* plus *Cronobacter* Detection LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For users of the Agilent Mx3005P instrument, Click "Instrument → Filter Set Gain Settings" to open the Filter Set Gain Settings dialog box. For all channels (FAM, HEX, ROX and Cy5), the Filter Set Gain Setting must be modified to "x1".

2.2.2 Preparation of the PCR Mix

2.2.2.1 For PCR Analysis Using DNA Isolated by foodproof DNA Extraction Methods and Real-Time PCR Cyclers Other than the BAX System Q7 Instrument.

Use this protocol to prepare a 25 µL standard reaction.

Always wear gloves when handling strips or caps. Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors.

Note: The PCR tube strips containing the lyophilized reagents must be stored in the provided aluminum bag with silica gel pads to avoid liquid absorption.

1. Remove the needed number of PCR tube strips from the aluminum bag. Use scissors or a scalpel to cut the strips apart. Tightly seal the bag afterward and store at the recommended conditions.
2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. Check that the reagent pellets are at the bottom of the tubes. If not, briefly centrifuge or flick the pellets to the bottom before proceeding.
3. Carefully uncap the tube strips and discard the cap strips.

   Note: To avoid unwanted liquid absorption, open strips only shortly before filling. Do not leave strips open for extended periods of time.
4. Pipet samples into each PCR tube:

   - For the samples of interest, add 25 µL of DNA extract from foodproof lysis kits or 30 µL of DNA extract from BAX Prep lysis kit into each PCR tube (if using less volume, add PCR-grade H_2O to achieve 25 µL or 30 µL, respectively).
   - For the negative control, add 25 µL of PCR-grade H_2O (vial 3, colorless cap).
   - For the positive control, add 25 µL of foodproof *Salmonella* plus *Cronobacter* Control Template (vial 2, purple cap).

   Note: To reduce the risk of cross-contamination, it is recommended to prepare only one PCR tube strip at a time.
5. Seal the PCR tube strips accurately and tightly with the colorless cap strips.
6. Mix thoroughly using a vortex centrifuge.

   Note: Hygiena Diagnostics GmbH recommends vortex centrifuges Multispin MSC-3000 for PCR-strips or vortex centrifuge CVP-2 for PCR-plates. Dedicated protocols are available for these centrifuges.

   Alternatively resuspend the pellet by manual mixing. This may be achieved by cautiously pipetting the sample up and down multiple times during Step 4 or flipping the tube strips after sealing while pressing down the cap strip.
7. Spin the PCR tube strips for 30 seconds at 150 – 200 x *g* in a suitable centrifuge.

   Note: If your centrifuge exceeds 200 x *g*, do not centrifuge for more than 5 seconds. Avoid centrifugal forces exceeding 1,000 x *g*!
8. Place the samples in your PCR cycler and run the program as described above.

   Note: For LightCycler 480 instruments, a special adapter (Product No. MIS230005) is necessary.

   For some PCR instruments, the PCR strips should be placed in a balanced order in the cycler block. For example, two strips can be placed in columns 1 and 12.

2.2.2.2 For PCR Analysis Using DNA Isolated by BAX Prep Lysis Kit or foodproof StarPrep Three (single tube and 8-tube strips) Lysis and the BAX System Q7 Instrument.

The BAX System Q7 must have HEX calibration. If you have questions about this, contact Technical Support: techsupport@hygiena.com.

1. Start or restart the BAX System Q7 instrument and computer workstation.
2. Open the BAX Q7 Software.
3. Select sample positions.
4. Select **foodproof – RT Sal spp + Cronobacter** in the Target dropdown menu.

   Note: To make this target available, in the BAX Q7 software, go to 'View' > 'Select Target', and select 'foodproof – RT Sal spp + Cronobacter' to move it to the 'Targets Available for Selection' table.
5. Click the **Apply** button.
6. Place the PCR tube strips from the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit in a suitable PCR tube rack according to your rack file. Check that the reagent pellets are at the bottom of the tubes. If not, briefly flick the pellets to the bottom before proceeding. Remove the caps from the first strip of tubes.

   Note: Reagent pellets must be hydrated with lysate or controls and re-sealed immediately after removing the caps from the PCR tubes.
7. Transfer 30 µL of lysate from BAX Prep lysis or 25 µL of lysate from StarPrep Three lysis into the PCR tubes, then seal with 8-cap strips. Resuspend the pellet after sealing by mixing thoroughly. Repeat with remaining strips of PCR tubes until all lyophilized pellets have been hydrated.
8. Add PCR controls: Transfer 25 µL of PCR-grade H_2O (negative control; vial 3, colorless cap) and 25 µL of Control Template (positive control; vial 2, purple cap) into respective PCR tubes and seal with 8-cap strips. Resuspend pellet after sealing by mixing thoroughly.
9. Briefly spin PCR tube strips in a suitable centrifuge, e.g., for 5 seconds at 500 – 1,000 x *g*.
10. Select **OPERATION > RUN FULL PROCESS** from the menu.
11. Save the rack file.
12. Follow the screen prompts.

2.3 Data Interpretation

2.3.1 Review Results for Real-Time PCR Instrument Other than BAX System Q7 Instrument

The amplification of the *Salmonella*-specific DNA region is analyzed in the fluorescence channel suitable for FAM-labeled probe detection. The amplification of the *Cronobacter*-specific DNA region is analyzed in the fluorescence channel suitable for HEX-labeled probe detection, and the specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for ROX-labeled probe detection.

Review the results from FAM (*Salmonella*), HEX (*Cronobacter*) and ROX (Internal Control) channels for each sample and ensure that each positive result has an amplification curve. In case no amplification curve is observed, the result is considered negative. Interpret the results as described in the table below.

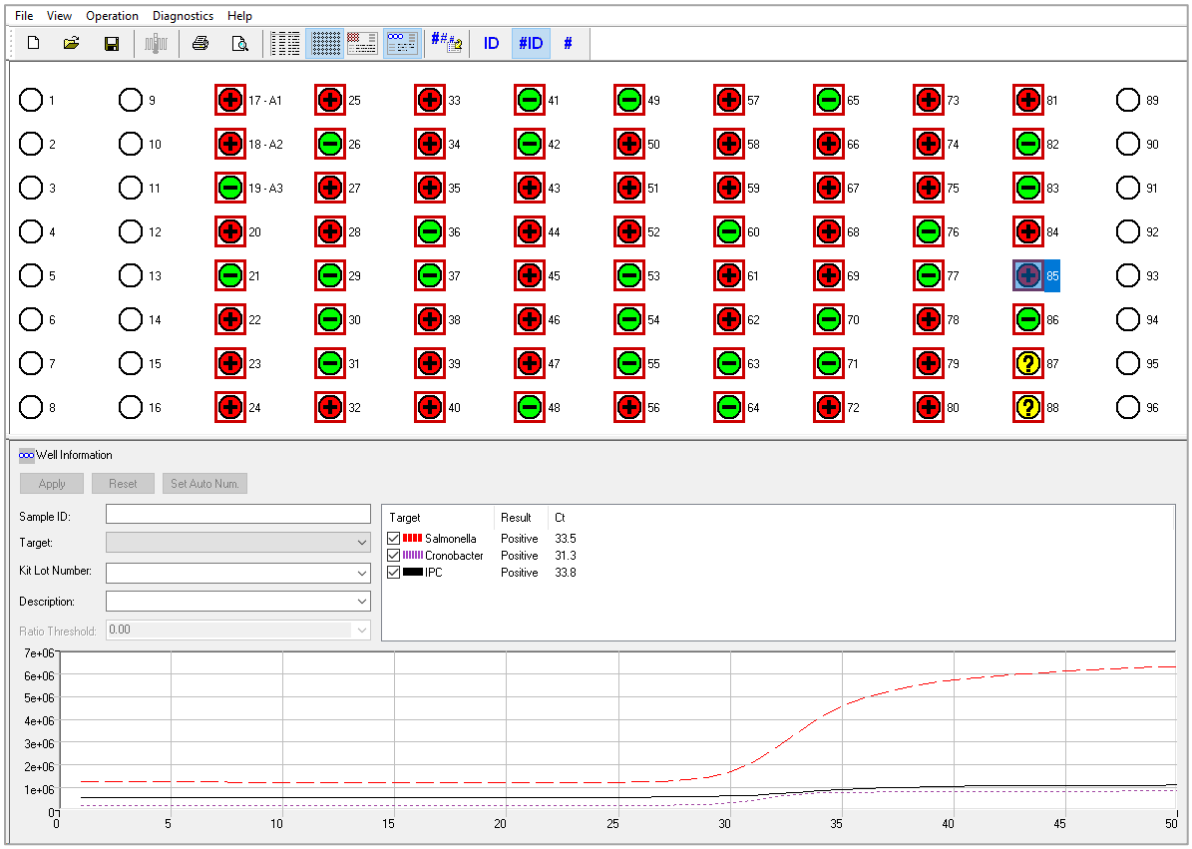
FAM	HEX	ROX	Result Interpretation
Positive	Positive	Positive or Negative	Positive for *Salmonella* spp. and *Cronobacter* spp.
Positive	Negative	Positive or Negative	Positive for *Salmonella* spp. and negative for *Cronobacter* spp.
Negative	Positive	Positive or Negative	Positive for *Cronobacter* spp. and negative for *Salmonella* spp.
Negative	Negative	Positive	Negative for *Salmonella* spp. and *Cronobacter* spp.
Negative	Negative	Negative	Invalid

Note: A prerequisite for the unambiguous discrimination of the targets in this multi-color experiment is a suitable calibration of the PCR instrument for FAM, HEX and ROX channels. Please refer to the operation manual of your real-time PCR cycler for further information.

Note: When using PCR instruments that are calibrated for VIC instead of HEX (e.g., ABI 7500 fast), signal crosstalk from the FAM to the VIC channel might occur. In some cases, a sample with a positive result for *Salmonella* (positive signal in the FAM channel) also has a weak fluorescence curve in the VIC channel that might be caused by signal crosstalk. Samples positive for *Salmonella* are only positive for *Cronobacter* too, when the maximum fluorescence value of the sample in the VIC channel reaches at least 25 % of the fluorescence value of the Control Template.

2.3.2 Review Results for BAX System Q7 Instrument

1. Qualitative results are displayed as a grid of color-coded icons in the top half of the screen.
2. Ensure that each positive result has an amplification curve. In case no amplification curve is observed, the result is considered negative.
3. Check Ct values for *Cronobacter* positive samples. If the Ct value in the HEX channel is ≥ 40, the result is considered negative.



Green (-)	=	Negative for target organism
Red (+)	=	Positive for target organism
Yellow (?)	=	Indeterminate result*
Yellow (?) with red slash	=	Signal error*

*Refer to the troubleshooting section in the BAX System Q7 User Guide

3. Troubleshooting

For troubleshooting results on the BAX Q7 System, refer to the BAX System User Guide.

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	• Set Channel settings to FAM, HEX or ROX.
	Pipetting errors.	• Check for correct reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	• Check the cycle programs.
No signal increase in the ROX channel is observed.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Use a recommended DNA sample preparation kit to purify template DNA. • Dilute samples or pipet a lower amount of sample DNA (e.g., 20 µL of PCR-grade H_2O with 5 µL of foodproof lysate or 20 µL PCR-grade H_2O with 10 µL of BAX lysates).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof *Salmonella* plus *Cronobacter* Detection lyophilized PCR mix at 2 to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	• Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline.	Resuspension of lyophilized PCR mix not complete.	• Always resuspend lyophilized PCR mix thoroughly.
Negative control samples are positive.	Carryover contamination.	• Exchange all critical solutions. • Repeat the entire experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carryover contamination. • Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Resuspended PCR mix is still in the upper part of the vessel.	• Always centrifuge PCR strips.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the vessels and seal.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	• Always store the lyophilized PCR mix in the aluminum bag with the silica gel pad • Open strips shortly before filling.
No confirmation by cultural method.	Signal due to DNA from dead cells.	• Dilute 100 µL primary enrichment in 10 mL new broth. Enrich for 16 – 24 h at 37 ± 1 °C, and retest the sample by PCR. If signal is due to dead cells, the new result will be negative or the Ct value will be higher (≥3 Ct) than the first result.

4. Additional Information on This Product

4.1 How This Product Works

The foodproof *Salmonella* plus *Cronobacter* Detection LyoKit provides all necessary reagents and a control template for reliable interpretations of results. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included. A hydrolysis probe was designed to bind specifically the IC, allowing detection in the ROX channel, whereas *Salmonella* DNA is detected in the FAM channel and *Cronobacter* DNA in the HEX channel.

In case of a negative result due to inhibition of amplification by the sample DNA of interest, the amplification of the IC is suppressed as well. A negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of *Salmonella* and *Cronobacter* in the sample.

The foodproof *Salmonella* plus *Cronobacter* Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of *Salmonella* and *Cronobacter* DNA. Primers and probes provide specific detection of *Salmonella* and *Cronobacter* DNA in food and environmental samples. The described performance of the kit is guaranteed for use on only the real-time PCR instruments listed above.

4.2 Test Principle

1. Using the kit's sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify fragments of specific sequences for the targets: *Salmonella* and *Cronobacter* spp.
2. The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probe due to the 5´-nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5´-end with a reporter fluorophore and at the 3´-end with a quencher.
3. During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal sequence of the amplicon and is cleaved by the 5’ nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.
4. The PCR instrument measures the emitted fluorescence of the reporter dye.

4.3 Prevention of Carryover Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions, and the pretreatment of all successive PCR mixtures with the heat-labile UNG. The UNG cleaves DNA at any site where a deoxyuridine residue has been incorporated. The resulting abasic sites are hydrolyzed due to the high temperatures during the initial denaturation step and can no longer serve as PCR templates. The heat-labile UNG is inactivated during the initial denaturation step. Native DNA (e.g., the isolated *Salmonella* or *Cronobacter* genomic DNA) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit, decontamination can be achieved with the provided reagents.

4.4 Background Information

In Regulation (EC) 2073/2005, the European Commission states that *Salmonella* and *Cronobacter* are the microorganisms of greatest concern in infant formula, formula for special medical purposes and follow-on formula. The lethality rate caused by neonatal *Cronobacter* infections is between 40 - 80%.

The foodproof *Salmonella* plus *Cronobacter* Detection LyoKit uses the same primer and probes for the specific detection of *Salmonella* spp. as the foodproof *Salmonella* Detection Kit (KIT230049) and for *Cronobacter* spp. as the foodproof *Enterobacteriaceae* plus *Cronobacter* Detection Kit (KIT230043), which are validated according to ISO 16140 (MicroVal certificates 2011-LR 40/42 and 2007-LR 08091920).

4.5 Quality Control

The foodproof *Salmonella* plus *Cronobacter* Detection LyoKit is function tested using the LightCycler 480 System (KIT230131, LP) and the Mx3005P (KIT230132, RP).

5. Supplementary Information

5.1 Ordering Information

Hygiena offers a broad range of reagents and services. For a complete overview and for more information, visit our website at www.hygiena.com.

5.2 License Notice

The purchase price of this product includes limited, nontransferable rights under US Patent No. 7,687,247 owned by Life Technologies Corporation to use only this amount of the product to practice the claims in said patent solely for activities of the purchaser for bioburden testing, environmental testing, food testing, or testing for genetically modified organisms (GMO) in accordance with the instructions for use accompanying this product. No other rights are conveyed, including no right to use this product for *in vitro* diagnostic, therapeutic, or prophylactic purposes. Further information on purchasing licenses under the above patent may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008.

Email: outlicensing@lifetech.com.

5.3 Trademarks

foodproof® is a registered trademark of Hygiena Diagnostics GmbH. Hygiena® is a registered trademark of Hygiena.

Other brand or product names are trademarks of their respective holders.

5.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers: R 602 57-1 (KIT230131), R 602 57--2 (KIT230132), and R 602 57-3 (KIT230133).

5.5 Contact and Support

If you have questions about this or any other product of Hygiena Diagnostics GmbH, contact our Technical Support staff (for details see www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. Contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

6. Change Index

Version 1, May 2019
First version of the package insert.

Version 2, May 2022
Order no. R 602 57-3 (DP) added.

Revision A, November 2023
Rebranding and new layout.
Specifications on data analysis.
Change document tracking number:
(R 602 57 20 -> INS-KIT230131-32-33-REVA).

Revision B, September 2024
Revision of paragraph 1.4 Additional Required Equipment and Reagents.
Additional information in 2.1.1 Precaution: Comply with Good Laboratory Practices.
Additional information in 1.5 Applicability Statement: Overview of the NF VALIDATIONS by AFNOR Certification.
Introduction of Appendix 1.
Introduction of NF VALIDATION logo.
Removal of StarPrep One kits from the extraction methods
Removal of software versions

Revision C, May 2025
Added AFNOR Certificate number
Additional information about BAX Q7 program set up in 2.2.1
Minor edits to test portion size in Table 1
Additional information in Table 1 footnote for pre-warming of enrichment broth
Additional information in Confirmation section of Appendix 1
Updated the name of the BAX Prep Lysis Kit
Updated the Technical Support email address

Appendix 1

Certified NF VALIDATION studies by AFNOR Certification
QUA 18/12-12/24 Detection method for *Salmonella* spp. and
QUA 18/13-12/24 Detection method for *Cronobacter* spp.
Validation Tables for the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit

The following tables show the enrichment conditions for infant formula (with and without probiotics, ingredients) and environmental samples (excluding primary production stage samples) as well as DNA extraction and PCR analysis procedures that have been approved in NF Validation studies by AFNOR Certification; one study for each target of this multiplex PCR kit (QUA 18/12-12/24 for detection of *Salmonella* spp. and QUA 18/13-12/24 for detection of *Cronobacter* spp.).

Table 1. Matrices and Enrichment Conditions Approved by AFNOR Certification

Category	Type	Test Portion Size*	Enrichment Broth†	Dilution Factor	Enrichment Time and Temperature	Sample Preparation
Infant formula with or without probiotics and ingredients	Probiotic infant formula	Up to 375 g	Prewarmed BPW + vancomycin (10 mg/L)	1:10	16 - 24 h 37 ± 1 °C	ISO 6887-1 ISO 6887-5
	Non-probiotic infant formula	Up to 375 g	Prewarmed BPW	1:10	16 - 24 h 37 ± 1 °C	ISO 6887-1 ISO 6887-5
	Ingredients	Up to 375 g	Prewarmed BPW	1:10	16 - 24 h 37 ± 1 °C	ISO 6887-1 ISO 6887-5
Production environmental samples (excluding primary production stage samples)	Surfaces	Swabs or sponges	BPW	10 mL or 100 mL	16 - 24 h 37 ± 1 °C	ISO 18593
	Vacuum dusts from non-probiotic production sites	Up to 200 g	Prewarmed BPW	1:10	16 - 24 h 37 ± 1 °C	ISO 6887-1 ISO 6887-4

* In the scope of NF VALIDATION, test portions weighing more than specified have not been tested.

† Prewarmed enrichment broth is required for large test portions according to the ISO 6887 series rules. Therefore, it is required to comply with the temperature conditions indicated in the technical specification. In particular, you must verify that the prewarming temperature of the enrichment broth reaches the required temperature. The preparation time of the samples, i.e., the time between the enrichment broth prewarming step and the beginning of the food sample incubation step, does not exceed 45 minutes. Ventilated incubators are recommended for incubation.

However, according to the ISO 6687 rules, for the usual 25 g test portion size for *Salmonella* screening and the usual 10 g test portion size for *Cronobacter* screening, no prewarming of the enrichment is required.

Table 2. DNA Extraction Methods and PCR Analysis Approved by AFNOR Certification

DNA Extraction[1] Kit and Protocol	Tube Format	Volume of Enrichment Culture for DNA Extraction	Volume of DNA Extract for PCR Analysis	Validated Real-Time PCR Instruments
foodproof StarPrep Three Kit Extraction Procedure A: STANDARD	single tube	100 µL	25 µL	LightCycler 480 (Roche Diagnostics) and BAX System Q7 (Hygiena)
foodproof StarPrep Three 8-Strip Kit Extraction Procedure A: STANDARD	8-tube strips	100 µL	25 µL	
BAX Prep Lysis Kit	8-tube strips	20 µL	30 µL	

[1] Before DNA extraction, it is possible to store enrichment cultures for 72 hours at 5 ± 3 °C.

For further information on the DNA extraction procedures above, please refer to the appropriate Hygiena Diagnostics GmbH package inserts by following this link: www.hygiena.com/dna-rna-extraction-kits.

Confirmation

In the context of NF validation technical rules, all samples identified as positive by the foodproof *Salmonella* plus *Cronobacter* method must be confirmed starting from a colony. The reference culture methods according to ISO 6579-1 for *Salmonella* standard and ISO 22964 for *Cronobacter* standard were used.

In the context of the ISO rules, it is possible to use one of the following options on isolated colonies:

- Any relevant ISO 16140-6 validated confirmation method
- According to the ISO 7218 standard, any alternative method based on a different measurement principle than the principle used in the detection or using different markers (e.g., different antibodies, primers) if properly validated (e.g., ISO 16140 validation)

In the event of discordant results (presumptive positive with the alternative method, not confirmed by the ISO method(s) mentioned above) the laboratory must use adequate methods to ensure the validity of the result obtained.

Note: According to the ISO 7218 standard, pure cultures should be used for biochemical and serological confirmation, although it is possible to conduct these on single colonies from selective agar plates in some cases (e.g., ISO 6579-1). For molecular amplification formats, the use of mixed cultures may be acceptable if sufficient target organism DNA is present. For more information, including the end of validity of the NF VALIDATION certifications, please refer to certificates QUA 18/12-12/24 and QUA 18/13-12/24 available on the website http://nf-validation.afnor.org.

Hygiena®
Camarillo, CA 93012
USA
techsupport@hygiena.com

Manufactured by
Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam
Germany
www.hygiena.com