



foodproof<sup>®</sup>

# ***Salmonella*** **Detection Kit**

## **PRODUCT INSTRUCTIONS**

Documentation for the qualitative detection of *Salmonella* DNA

Product No. KIT230049 / KIT230050

foodproof®

## **Salmonella Detection Kit**

Product No. KIT230049

Kit for 96 reactions for a  
maximum of 94 samples

Product No. KIT230050

Kit for 480 reactions for a  
maximum of 470 samples

Store kit at -25 to -15 °C

For testing of food  
and environmental samples

Approvals:



**PRODUCT INSTRUCTIONS**

Revision A, May 2024

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## OVERVIEW

# 1. OVERVIEW

## 1.1 General Information

### Number of Reactions

The kit is designed for 96 reactions or 480 reactions, respectively, with a final reaction volume of 25  $\mu$ L each. Up to 94 samples or 470 samples plus positive and negative control can be analyzed.

### Storage and Stability

Store all components at -25 to -15 °C. They are guaranteed to be stable through the expiration date printed on the label. Opening of the kit does not shorten the expiration date.

## 1.2 Applicability

The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM and a VIC/HEX detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480, LightCycler® 96 (Roche Diagnostics), Applied Biosystems™ 7500 Fast (Thermo Scientific) and Mx3005P® (Agilent).

The foodproof® *Salmonella* Master Mix is sequence-specific for a highly conserved gene found in all subgroups of *Salmonella*. Inclusivity has been tested in several internal and external studies (AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> (AOAC RI *PTM*<sup>SM</sup>) and MicroVal®) with more than 700 strains of *Salmonella enterica* (all subspecies) and *Salmonella bongori* comprising members of all O-serogroups according to White-Kauffmann-Le Minor scheme 2007. Exclusivity was determined during the above mentioned studies using more than 50 species of closely related organisms or organisms occurring in the same habitat.

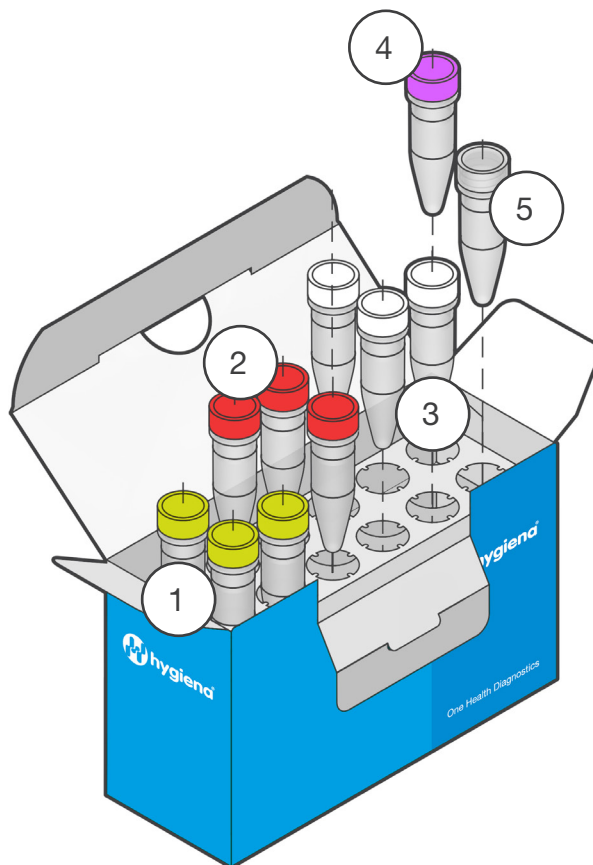
A relative detection limit of 1 to 10 cells per 25 g sample can be achieved with all kinds of foods. The foodproof *Salmonella* Detection Kit detects down to  $10^3$  -  $10^4$  CFU/mL in enrichment cultures (depending on which sample preparation kit is used: foodproof ShortPrep® I Kit, foodproof StarPrep® One Kit or foodproof Magnetic Preparation Kits I or IV).

## OVERVIEW

### 1.3 Kit Contents

A schematic representation of the foodproof *Salmonella* Detection Kit with all its components.

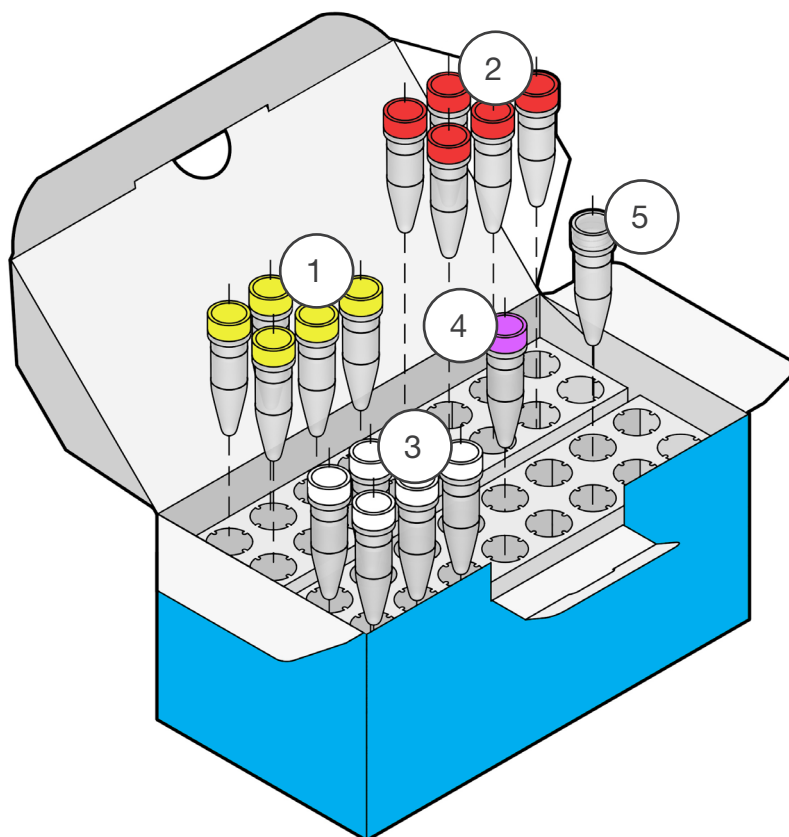
#### KIT 2300 49:



	Component	Details
1	Master Mix (yellow cap)	3 x 600 $\mu$ L, ready-to-use primer and hydrolysis probe mix specific for parameter DNA and the parameter-specific Internal Control (IC). Store at -25 to -15 $^{\circ}$ C. <b>Avoid repeated freezing and thawing! Protect from light!</b>
2	Enzyme Solution (red cap)	3 x 32 $\mu$ L, contains Taq DNA Polymerase and Uracil-DNA Glycosylase (heat-labile) for prevention of carry-over contamination. Store at -25 to -15 $^{\circ}$ C.
3	Internal Control (white cap)	3 x 32 $\mu$ L, contains a stabilized solution of plasmid DNA and a yellow dye for better visualization. For use as an internal amplification control. Store at -25 to -15 $^{\circ}$ C. Optional: After first thawing, store at 2 to 8 $^{\circ}$ C for up to one month.
4	Control Template (purple cap)	1 x 150 $\mu$ L, contains a stabilized solution of DNA. For use as a PCR run positive control. Store at -25 to -15 $^{\circ}$ C. Optional: After first thawing, store at 2 to 8 $^{\circ}$ C for up to one month.
5	Negative Control (transparent cap)	1 x 1 mL, contains PCR-grade water. For use as a PCR run negative control. Store at -25 to -15 $^{\circ}$ C. Optional: After first thawing, store at 2 to 8 $^{\circ}$ C for up to one month.

## OVERVIEW

## KIT 2300 50:



	Component	Details
1	Master Mix (yellow cap)	5 x 1,800 $\mu$ L, ready-to-use primer and hydrolysis probe mix specific for parameter DNA and the parameter-specific Internal Control (IC). Store at -25 to -15 $^{\circ}$ C. <b>Avoid repeated freezing and thawing! Protect from light!</b>
2	Enzyme Solution (red cap)	5 x 96 $\mu$ L, contains Taq DNA Polymerase and Uracil-DNA Glycosylase (heat-labile) for prevention of carry-over contamination. Store at -25 to -15 $^{\circ}$ C.
3	Internal Control (white cap)	5 x 96 $\mu$ L, contains a stabilized solution of plasmid DNA and a yellow dye for better visualization. For use as an internal amplification control. Store at -25 to -15 $^{\circ}$ C. Optional: After first thawing, store at 2 to 8 $^{\circ}$ C for up to one month.
4	Control Template (purple cap)	1 x 250 $\mu$ L, contains a stabilized solution of DNA. For use as a PCR run positive control. Store at -25 to -15 $^{\circ}$ C. Optional: After first thawing, store at 2 to 8 $^{\circ}$ C for up to one month.
5	Negative Control (transparent cap)	1 x 1 mL, contains PCR-grade water. For use as a PCR run negative control. Store at -25 to -15 $^{\circ}$ C. Optional: After first thawing, store at 2 to 8 $^{\circ}$ C for up to one month.

**INSTRUCTIONS****2. INSTRUCTIONS****2.1 Required Material**

Most of the required equipment and reagents are available through Hygiena®. Please contact us for further information.



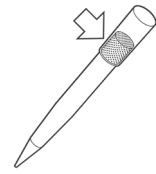
*Use a real-time PCR cyclor suitable for detection of respective probes.*

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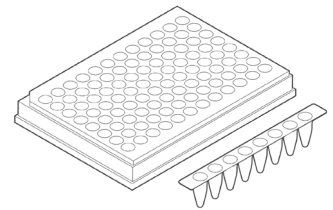
**Material**

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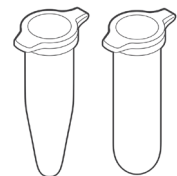
- Nuclease-free, aerosol-resistant pipette **filter tips**



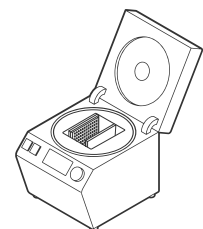
- Real-time PCR compatible **strips or plates** with optical cap or foil



- Sterile **reaction tubes** for preparing PCR mixes and dilutions



- PCR strip or plate centrifuge



## INSTRUCTIONS

### 2.2 Precautions and Preparations

The kit provides all reagents required for the PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease, carry-over or cross-contamination:

- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol barrier pipette tips.
- To avoid carry-over 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 carry-over contamination. Use a PCR hood for all pipetting steps.
- Sample Material:** Use any sample material suitable for PCR in terms of purity, concentration and absence of inhibitors.
- DNA Extraction:** We provide sample preparation kits suitable for all kind of food samples and primary production stage samples.
- Positive Control:** Always run a positive control with the samples. Use the provided control DNA (Control Template) or a positive sample preparation control.
- Negative Control:** Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water. 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.
- Confirmation:** If required, positive results may be confirmed by appropriate methods (e.g., reference method).
- Waste Disposal:** All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For more information, e.g., proper disposal of unused chemicals, please refer to the appropriate safety data sheet (SDS).



*Keep the PCR mix away from light.*

For more information, please refer to the appropriate safety data sheet (SDS). The SDS is available online at [www.hygiena.com/sds](http://www.hygiena.com/sds).



## INSTRUCTIONS

### 2.3 Enrichment and DNA Extraction

The foodproof *Salmonella* Detection Kit is intended for the rapid detection of *Salmonella* DNA isolated from enrichment cultures prepared by valid methods with all relevant kinds of samples that are potentially contaminated with *Salmonella*. This method is intended for use in a laboratory setting. The kit must not be used in diagnostic procedures.

General procedure: Pre-enrichment broth and temperature according to ISO 6579 or BAM (Chapter 5) or USDA for  $20 \pm 2$  h. Sub-cultivation 1:10 in pre-warmed brain heart infusion broth (BHI; e.g., 1 mL sample + 9 mL broth) for 3 h at 37 °C is recommended. Other suitable, validated enrichment procedures can also be used.

#### Recommended DNA extraction kits:

- ⇒ KIT230175 /76 - StarPrep One Kit / large (suitable for most matrices)
- ⇒ KIT230179 - Magnetic Preparation Kit I (for an automated extraction)
- ⇒ KIT230184 - Magnetic Preparation Kit IV (for an automated extraction)
- ⇒ KIT230170 - ShortPrep I Kit (for a very quick extraction)

#### 2.3.1 Certified Methods

The kit is AOAC RI *PTM* program validated with the following foods: chicken breast, cocoa powder, coconut, cumin, dough, dry pet food, egg powder, food dye, frankfurter sausage, ice cream, Lyoner sausage, milk chocolate, milk powder, minced meat, pasta, sliced cabbage, smoked fish, watermelon, wet pet food, white pepper and environmental samples (AOAC RI certificate No. 120301). The validation was done on a LightCycler 1.5 from Roche Diagnostics.

AOAC RI (Certificate no. 120301)

Validation with the foodproof ShortPrep I Kit, KIT230170 (S 400 01) for DNA extraction according to FDA-BAM:

- White pepper and cumin: enrichment in TSB for  $24 \pm 2$  h at 35 °C, sub-cultivation in 1/10 BHI, 3 h at 37 °C
- Milk powder: enrichment in brilliant green water for  $24 \pm 2$  h at 35 °C, sub-cultivation in 1/10 BHI, 3 h at 37 °C
- Cocoa: enrichment in low fat milk for  $24 \pm 2$  h at 35 °C, sub-cultivation in 1/10 BHI, 3 h at 37 °C
- Egg powder, coconut, sausages, smoked fish, ice cream, watermelon, sliced cabbage, food dye, pasta, dough, wet and dry pet food: enrichment in lactose broth for  $24 \pm 2$  h at 35 °C, sub-cultivation in 1/10 BHI, 3 h at 37 °C

## INSTRUCTIONS

In a harmonized MicroVal / AOAC RI validation (MicroVal projects 2011LR40 and 2011LR42, AOAC RI certificate no. 120301), the kit is validated in combination with the foodproof Magnetic Preparation Kit I (KIT230179) for automated sample preparation and the foodproof StarPrep One Kit (KIT230175) for manual sample preparation. The validation was done with the following food categories: beef meat, chocolate and bakery products, egg products, feed samples, meat and meat products, milk and dairy products and primary production stage samples (PPS). The validation was done with the following PCR instruments: LightCycler 480 II (software version 1.5.1) from Roche, CFX96 from Bio-Rad (software version CFX Manager 2.0) and Mx3005P from Agilent (software version MX4.1d). To be in compliance with the actual standards, a renewal of the MicroVal projects 2011LR40 and 2011LR42 was done according to ISO 16140-2. The table below shows an overview how the samples were processed within the latest study.

### Protocols MicroVal projects 2011LR40 / 2011LR42 – Renewal study

Category	Enrichment	Subcultivation	DNA Isolation	Confirmation
Meat and meat products/ milk and dairy/egg products/ feed samples	25 g + 225 mL BPW for 20 ± 2 h at 37 ± 1 °C	1 mL + 9 mL prewarmed BHI 3 to 4 h at 37 ± 1 °C (optional)	foodproof StarPrep One	According to ISO 6579- 1:2017
Cocoa and cocoa-containing products	25 g + 225 mL milk (UHT skimmed) + 0.018 g/L Brilliant green for 20 ± 2 h at 37 ± 1 °C	1 mL + 9 mL prewarmed BHI 3 to 4 h at 37 ± 1 °C (optional)	foodproof StarPrep One	According to ISO 6579- 1:2017
Beef meat	375 g + 3375 mL BPW for 20 ± 2 h at 37 ± 1 °C	1 mL + 9 mL prewarmed BHI 3 to 4 h at 37 ± 1 °C (optional)	foodproof StarPrep One	According to EN ISO 6579- 1:2017
Meat and meat products/ milk and dairy/egg products/ feed samples	25 g + 225 mL BPW for 19 to 20 h at 37 ± 1 °C	None	foodproof Magnetic Preparation Kit I	According to ISO 6579- 1:2017
Cocoa and cocoa-containing products	25 g + 225 mL milk (UHT skimmed) + 0.018 g/L Brilliant green for 19 to 20 h at 37 ± 1 °C	None	foodproof StarPrep One	According to ISO 6579- 1:2017
PPS	25 g + 225 mL BPW for 16 to 17 h at 37 ± 1 °C	0.1 mL + 10 mL RVS broth 16 to 24 h at 41.5 ± 1 °C	foodproof StarPrep One on 500 µL enrichment	According to ISO 6579- 1:2017

## INSTRUCTIONS

The foodproof *Salmonella* Detection Kit is MicroVal-validated according to ISO 16140-2:2016, certificate number 2011LR39.

In this study, the foodproof *Enterobacteriaceae* plus *Cronobacter* Detection Kit for pre-screening, followed by the foodproof *Salmonella* Detection Kit, were validated; both in combination with the foodproof Magnetic Preparation Kit IV (KIT230184) and Reagent D (KIT230001) for automated sample preparation. The validation was done according to section “MicroVal Protocol including the semi-automated DNA extraction” in the manual for the foodproof *Enterobacteriaceae* plus *Cronobacter* Detection Kit.

Manual DNA extraction was performed with the foodproof StarPrep One Kit (KIT230175 / 76) and Reagent D (KIT230001), according to the section “Protocol including the manual DNA extraction with the foodproof StarPrep One Kit” in the manual for the foodproof *Enterobacteriaceae* plus *Cronobacter* Detection Kit.

For the manual extraction procedure, the alternative method was performed on: infant formula and infant cereals, probiotics containing products, ingredients and environmental samples for the manual extraction procedure. For the automated DNA extraction procedure, the alternative method was validated to be applicable to the scope: infant formula and infant cereals as well as probiotic-containing products. The validation was performed in comparison to the ISO method for *Salmonella* (ISO 6579-1:2017). For the validation the LightCycler 480 II (software version 1.5.1) from Roche Diagnostics was used.

If the foodproof *Salmonella* Detection Kit is used in combination with the foodproof *Enterobacteriaceae* plus *Cronobacter* Detection Kit for pre-screening, please refer to the manual of the foodproof *Enterobacteriaceae* plus *Cronobacter* Detection Kit (KIT230043).

### Cultural Confirmation

Positive PCR results were confirmed with the ISO 6579 reference method for MicroVal validations and with the FDA-BAM (Bacteriological Analytical Manual Online, Chapter 5: *Salmonella*, Andrews H.W., Hammack S.T., December 2007 Edition) for AOAC RI validations, e.g., serologically by latex agglutination (*Salmonella* Test Kit, Oxoid DR1108A) and biochemically by using API 20E strips (bioMerieux 20100). For further information please visit the following web addresses: [www.iso.org](http://www.iso.org) or [www.fda.gov](http://www.fda.gov).

Within the MicroVal study LR39, all *Salmonella*-positive PCR results were culture-confirmed as described in ISO 6579-1:2017.

Within the MicroVal study 2011LR40 / 2011LR42, all *Salmonella*-positive PCR results were culture-confirmed as described in EN ISO 6579/A1 (2007) for PPS samples and according EN ISO 6579:2002 for food and feed samples.

## INSTRUCTIONS

### **Details of the confirmation procedure within the MicroVal studies**

In the context of the MicroVal validations, all presumptive positive samples must be confirmed. For this, 0.1 mL of the first enrichment in BPW was transferred into 10 mL RVS (incubate at 41.5 °C for 24 ± 3 h) and 1 mL enrichment was transferred into 10 mL MKTTn (incubate at 37 °C for 24 ± 3 h). 10 µL from MktTn and RVS were spread onto XLD and a chromogenic agar. For the PPS samples, MRVS was inoculated with the BPW enrichment (incubate at 41.5 °C for 24 ± 3 h or optionally additional 24 ± 3 h). Isolations were done onto XLD and a chromogenic agar. For all samples: Incubate the plates according to the manufacturer's instruction. Confirm 1 to 5 characteristic colonies using the ISO 6579 reference confirmation tests.

For the automated protocol, the isolation and confirmation steps were only done for RVS and XLD. For the PPS samples, the confirmation was additionally done by streaking 10 µL MOSSEL broth onto XLD and a chromogenic agar, followed by confirmation using the ISO 6579 reference confirmation tests.

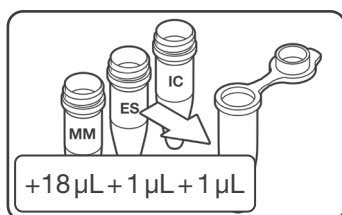
## INSTRUCTIONS

### 2.4 Procedure

This protocol describes how to perform the analysis of DNA extracts by real-time PCR.

#### 2.4.1 Workflow

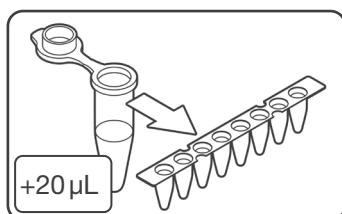
Thaw the solutions, mix by flicking the tubes four to five times and briefly spin vials in a microcentrifuge before opening.



#### 1. PREPARE PCR MIX

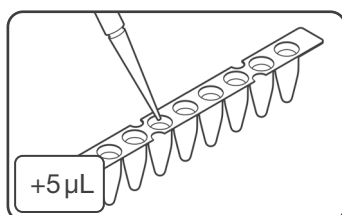
Add 18  $\mu\text{L}$  of Master Mix (yellow cap), 1  $\mu\text{L}$  Enzyme Solution (red cap) and 1  $\mu\text{L}$  Internal Control (white cap) for each reaction to a suitable tube (n samples + 2 controls + at least one additional reaction to cover pipetting loss).

Mix carefully but thoroughly by pipetting up and down.



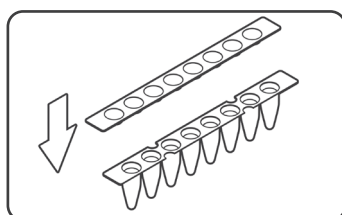
#### 2. ADD PCR MIX

Pipette 20  $\mu\text{L}$  of prepared PCR mix into each strip or plate well.



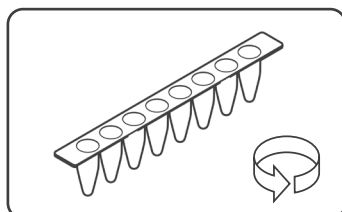
#### 3. ADD SAMPLES AND CONTROLS

Pipette 5  $\mu\text{L}$  of samples, negative control (colorless cap) or Control Template (purple cap) into respective wells.



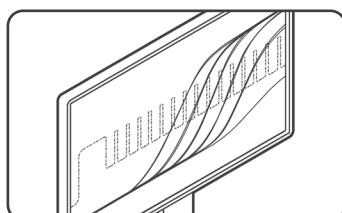
#### 4. SEAL

Seal strips/plate accurately.



#### 5. CENTRIFUGE

Briefly spin strips/plate in a suitable centrifuge.



#### 6. START REAL-TIME PCR RUN

Cycle samples as described in the program setup (2.4.2).

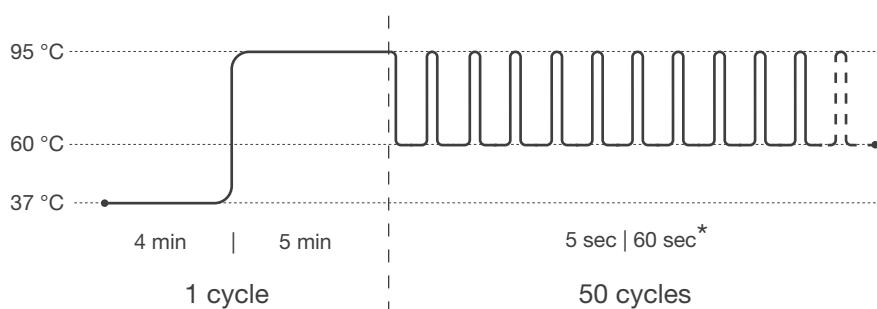
## INSTRUCTIONS

### 2.4.2 Program Setup

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

- FAM (*Salmonella*), and VIC (Internal Control).

As an alternative to VIC, HEX can be used. For the PikoReal® 24, Yakima Yellow has to be selected.



**Pre-incubation: 1 cycle**

Step 1: 37 °C for 4 min

Step 2: 95 °C for 5 min

**Amplification: 50 cycles**

Step 1 : 95 °C for 5 sec

Step 2\*: 60 °C for 60 sec

\* Fluorescence detection

For some real-time PCR instruments, the probe quencher as well as the usage of a passive reference dye has to be specified. This kit contains probes with TAMRA as quencher and no passive reference dye.

For users of the Agilent Mx3005P instrument: Click 'Instrument' and 'Filter Set Gain Settings' to open the Filter Set Gain Settings dialog box in which the gain settings may be viewed and modified. For FAM the Filter Set Gain Setting has to be modified to 'x1'.

### 2.4.3 Data Interpretation

Verify results of positive (Control Template) and negative controls (H<sub>2</sub>O), before interpreting sample results. Always compare samples to positive and negative controls. Review data from each channel and interpret results as described in the table.

FAM	VIC	Result Interpretation
+	+ or -	Positive for <i>Salmonella</i>
-	+	Negative for <i>Salmonella</i>
-	-	Invalid

## INSTRUCTIONS

### 2.5 Troubleshooting

Problem	Possible Cause	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	Set channel settings for respective dyes accordingly.
	Pipetting errors.	Check for correct reaction setup and repeat the PCR run. Always run a positive control along with your samples.
	No data acquisition programmed.	Check the cycle programs.
A sample shows no signals, including the internal control. Positive and negative control have proper signals.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	Use the recommended DNA sample preparation kit. Dilute samples 1:10 (e.g., 5 µL of sample and 45 µL of PCR-grade H <sub>2</sub> O).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	Store the reagents at -25 to -15 °C, protected from light. Avoid repeated freezing and thawing.
	Low initial amount of target DNA.	If possible, increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
	Reagents are not homogeneously mixed.	Mix reagents thoroughly before pipetting. Do not vortex enzyme solution.
Negative control samples are positive.	Carry-over contamination.	Exchange all critical solutions and DNA/RNA extraction reagents. Repeat the complete experiment with fresh batches of all reagents. Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies or changes abruptly during the run.	Insufficient centrifugation of the PCR strips or plate (e.g., PCR mix is still in the upper part of the vessel or bubbles are trapped in the mix).	Always centrifuge PCR strips or plate. Use the centrifuge models and settings recommended in these product instructions. Avoid the introduction of air bubbles during pipetting.
	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. Do not mark vessels on the outside of the tubes or directly on top of the reaction mix.

## INSTRUCTIONS

### 2.6 Support

If you have questions or experience any problems with our products, please contact us:



[www.hygiena.com/support](http://www.hygiena.com/support)

Our aim is to provide you with a solution as quickly and effectively as possible. We would also like you to contact us if you have any suggestions for improving the product or in case you would like to use our product for a different application. We highly value your feedback.



## ADDITIONAL INFORMATION

# 3. ADDITIONAL INFORMATION

## 3.1 Testing Principle

The foodproof kit 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 to the IC, allowing detection in the respective channel, whereas the target DNA is detected in another channel. In case of a negative result due to amplification inhibition of the sample DNA of interest, the amplification of the IC is suppressed as well, whereas a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of target DNA in the sample. The real-time PCR kit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of target DNA. Primers and probes provide specific detection of target DNA in food and environmental samples, including primary production stage samples. The described performance of the kit is guaranteed for use only on the real-time PCR instruments listed in Section 1.2: Applicability. For other instruments, please contact us.

### Step-by-Step Procedure

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 of target DNA.
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.

### Prevention of Carry-Over 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 target 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 this kit, decontamination can be achieved with the provided reagents.

## ADDITIONAL INFORMATION

### 3.2 Trademarks

**foodproof**<sup>®</sup>, **microproof**<sup>®</sup>, **vetproof**<sup>®</sup>, ShortPrep<sup>®</sup>, StarPrep<sup>®</sup>, RoboPrep<sup>®</sup> and LyoKit<sup>®</sup> are trademarks of Hygiena Diagnostics GmbH.

Hygiena<sup>®</sup> is a registered trademark of Hygiena.

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

### 3.3 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers:

R 302 27 and R 302 27 L.

### 3.4 Change Index

*Version 1, June 2008:*

First version of the package insert.

*Version 2, December 2008:*

New product name extension: 5'Nuclease

*Version 3, July 2010:*

Page 8: NOTE for users of the Agilent Mx3005P instrument added.

*Version 4, October 2010:*

Pages 1 and 4: Information about large version of the kit with 480 reactions added.

*Version 5, February 2022:*

Information regarding the MicroVal validation has been included.

Rebranding, new document layout and updated content.

*Revision A, May 2024:*

Updated rebranding: Logo, product numbers.

R 302 27 20 -> INS-KIT230049-50-REVA

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