



## **foodproof® *Salmonella* Enteritidis & Typhimurium Detection LyoKit**

### **Revision A, October 2023**

PCR system for the qualitative detection of *Salmonella* Enteritidis and *Salmonella* Typhimurium, including the monophasic variant (4,[5],12:i:-) of *Salmonella* Typhimurium, using real-time PCR instruments.

**Product No. KIT230106 (LP)**

**Product No. KIT230107 (RP)**

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

**Store at 2 to 8 °C**

For food testing purposes.

**FOR *IN VITRO* USE ONLY**



## Table of Contents

- 1. What this Product Does ..... 3
  - 1.1 Number of Tests ..... 3
  - 1.2 Storage and Stability ..... 3
  - 1.3 Kit Contents ..... 3
  - 1.4 Additional Equipment and Reagents Required ..... 4
  - 1.5 Applicability Statement ..... 4
- 2. How to Use this Product ..... 4
  - 2.1 Before You Begin ..... 4
    - 2.1.1 Precautions ..... 4
    - 2.1.2 Waste Disposal ..... 5
    - 2.1.3 Sample Material ..... 5
    - 2.1.4 DNA Extraction ..... 5
    - 2.1.5 Positive Control ..... 5
    - 2.1.6 Negative Control ..... 5
  - 2.2 Procedure ..... 6
    - 2.2.1 Program Setup ..... 6
    - 2.2.2 Preparation of the PCR Mix ..... 7
  - 2.3 Procedure for colony of *Salmonella* ..... 8
    - 2.3.1 Program Setup ..... 8
    - 2.3.2 Preparation of the PCR Mix ..... 9
  - 2.4 Data Interpretation ..... 10
- 3. Troubleshooting ..... 11
- 4. Additional Information on this Product ..... 12
  - 4.1 How this Product Works ..... 12
  - 4.2 Test Principle ..... 12
  - 4.3 Prevention of Carryover Contamination ..... 13
  - 4.4 Background Information ..... 13
  - 4.5 References ..... 13
  - 4.6 Quality Control ..... 13
- 5. Supplementary Information ..... 14
  - 5.1 Ordering Information ..... 14
  - 5.2 License Notice ..... 14
  - 5.3 Trademarks ..... 14
  - 5.4 Contact and Support ..... 14
  - 5.5 Reference Number ..... 14
- 6. Change Index ..... 14



## 1. What this Product Does

### 1.1 Number of Tests

The detection system is designed for 96 reactions with a final reaction volume of 25 µL each. 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 components as described in the following Kit Contents table.

### 1.3 Kit Contents

Component	Description	Contents, Function, Storage
foodproof® <i>Salmonella</i> Enteritidis & Typhimurium Detection LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat: <ul style="list-style-type: none"> <li>• KIT230106 (LP) with white low-profile tubes</li> <li>• KIT230107 (RP) with clear regular-profile tubes</li> </ul>	<ul style="list-style-type: none"> <li>• 96 prefilled reactions (lyophilized)</li> <li>• Ready-to-use PCR mix containing primer and hydrolysis probes specific for <i>Salmonella</i> Enteritidis and <i>Salmonella</i> Typhimurium DNA and the Internal Control (IC) as well as Taq DNA Polymerase and Uracil-DNA Glycosylase (UNG, heat-labile) for prevention of carry-over contamination.</li> <li>• For amplification and detection of <i>Salmonella</i> Enteritidis and <i>Salmonella</i> Typhimurium-specific sequences</li> <li>• Store at 2 to 8 °C in the aluminum bag (sealed)</li> <li>• <b>Protect from light and moisture!</b></li> </ul>
foodproof <i>Salmonella</i> Enteritidis & Typhimurium Detection Control Template	Vial 1 (purple cap)	<ul style="list-style-type: none"> <li>• 1 x 250 µL</li> <li>• Contains a stabilized solution of DNA</li> <li>• For use as a PCR positive control</li> <li>• Store at 2 to 8 °C</li> </ul>
H <sub>2</sub> O, PCR-grade	Vial 2 (colorless cap)	<ul style="list-style-type: none"> <li>• 2 x 1 mL</li> <li>• Nuclease-free, PCR-grade H<sub>2</sub>O</li> <li>• For use as a PCR negative control</li> </ul>
Cap strips	Plastic bag containing 8-cap strips	<ul style="list-style-type: none"> <li>• 12 x 8-cap strip</li> <li>• For use in real-time PCR after addition of samples</li> </ul>



## 1.4 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, HEX- and ROX-labeled probes and for low- or regular-profile strip tubes. If the strip tubes do not fit into the instrument, the samples should be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.
- Sample preparation kit options (choose one):
  - foodproof StarPrep Three Kit (Product No. KIT230187)
  - foodproof StarPrep One Kit (Product No. KIT230175)
  - foodproof Magnetic Preparation Kit I (Product No. KIT230180)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex centrifuge (choose one):
  - For PCR strips, Multispin MSC-6000 with the SR-32 Rotor
  - For PCR plates, CVP-2

## 1.5 Applicability Statement

The foodproof *Salmonella* Enteritidis & Typhimurium Detection LyoKit is intended for the rapid detection of *Salmonella* Enteritidis and *Salmonella* Typhimurium isolated from enrichment cultures prepared by valid methods and inoculated with all relevant kinds of foods and primary production samples that are potentially contaminated with *Salmonella* Enteritidis or *Salmonella* Typhimurium.

The kit must not be used in diagnostic procedures.

The kit described in this Instruction Manual has been developed for real-time PCR instruments with FAM, HEX and ROX detection channels. The performance of the kit was tested with the following real-time PCR instruments: LightCycler<sup>®</sup> 480 (Roche Diagnostics), LightCycler<sup>®</sup> 96 (Roche Diagnostics), Mx3005P<sup>®</sup> (Agilent Technologies), Applied Biosystems<sup>®</sup> 7500 Fast (Applied Biosystems), and PikoReal 24<sup>™</sup> (Thermo Scientific), CFX96<sup>™</sup> (BIO-RAD).

**Note:** Color Compensation (Color Compensation Set 3; Product No. KIT230005) is necessary and will be supplied by Hygiena Diagnostics for users of the LC 480 Systems I and II. Contact Hygiena Diagnostics for further information.

## 2. How to Use this Product

### 2.1 Before You Begin

#### 2.1.1 Precautions

Detection of *Salmonella* Enteritidis and Typhimurium DNA using the foodproof *Salmonella* Enteritidis & Typhimurium 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. 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., pipettes, pipette tips and 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* Enteritidis & Typhimurium Detection Lyophilized PCR Mix away from light and moisture.**

### **2.1.2 Waste Disposal**

Place any waste and biohazard material potentially contaminated with pathogenic bacteria in an appropriate contaminated waste bag. The bag should be treated and disposed of according to local regulations.

### **2.1.3 Sample Material**

Use any sample material suitable for PCR in terms of purity, concentration and absence of inhibitors. For preparation of genomic DNA from food or primary production samples, refer to the corresponding product package inserts of a suitable sample preparation kit (see “*Additional Equipment and Reagents Required*”).

### **2.1.4 DNA Extraction**

Hygiena Diagnostics provides sample preparation kits suitable for all kinds of foods and primary production samples (see “*Additional Equipment and Reagents Required*”). For more product information, visit [www.hygiena.com](http://www.hygiena.com).

### **2.1.5 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* Enteritidis & Typhimurium Detection Control Template (vial 1, purple cap)] or with a positive sample preparation control.

### **2.1.6 Negative Control**

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H<sub>2</sub>O, PCR-grade (vial 2, 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.



## 2.2 Procedure

### 2.2.1 Program Setup

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

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 in step 2

#### Notes:

- For some real-time PCR instruments, the type of probe quencher as well as the use of a passive reference dye must be specified. The foodproof *Salmonella* Enteritidis & Typhimurium Detection LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For the PikoReal 24, Yakima Yellow (YY) has to be chosen, instead of HEX.
- For users of the Agilent Mx3005P instrument: Click "Instrument → Filter Set Gain Settings" to open the Filter Set Gain Settings dialog box. For FAM, the Filter Set Gain Setting must be modified to FAM "x2", HEX to "x2" and ROX to "x1".
- For CFX 96 Analysis Software: 'Apply Fluorescence Drift Correction' in 'Settings' → 'Baseline Settings' must be activated.



### 2.2.2 Preparation of the PCR Mix

Proceed as described below to prepare a 25 µL standard reaction.

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

**Note:** The PCR strips must be stored in the provided aluminum bag with the silica gel pads to prevent liquid absorption.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or a scalpel to cut the strips apart. Tightly seal the bag afterwards and store at the recommended conditions.
2. Place the PCR tube strips 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 remove and discard the caps from the tube strips.

**Note:** To avoid unwanted liquid absorption, open strips shortly before filling. Do not leave strips open for extended periods of time.

4. Pipet 25 µL of sample into each PCR vessel:
  - For the samples of interest, add 25 µL of sample DNA (if using less volume, add PCR-grade H<sub>2</sub>O up to 25 µL).
  - For the negative control, add 25 µL of H<sub>2</sub>O, PCR-grade (vial 2, colorless cap).
  - For the positive control, add 25 µL of foodproof *Salmonella* Enteritidis & Typhimurium Detection Control Template (vial 1, purple cap).

**Note:** To reduce the risk of cross-contamination, it is recommended to prepare one PCR tube strip at a time.

5. Seal the PCR vessels accurately and tightly with the colorless cap strips.
6. Mix thoroughly using a vortex centrifuge.

**Note:** Hygiena Diagnostics recommends vortex centrifuge 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 1000 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. MIS230006) 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.



## 2.3 Procedure for colony of *Salmonella*

### 2.3.1 Program Setup

The following procedure is optimized for rapid confirmation and identification of presumptive *Salmonella* colonies obtained in microbiological culture methods (e.g., ISO 6579) for a real-time PCR instrument with detection channels for FAM (*Salmonella* Enteritidis), HEX (*Salmonella* Typhimurium) and ROX (Internal Amplification Control). Program the PCR instrument before preparing the samples. Use the following real-time PCR protocol for the foodproof *Salmonella* Enteritidis & Typhimurium Detection LyoKit. For details on how to program the experimental protocol, see the Instrument Operator's Manual for your real-time PCR cyclor.

Pre-incubation 1 cycle

Step 1: 37°C for 4 minutes

Step 2: 95°C for 5 minutes

Amplification 30 cycles

Step 1: 95°C for 5 seconds

Step 2\*: 60°C for 60 seconds

\* Fluorescence detection in step 2

#### Notes:

- For some real-time PCR instruments, the type of probe quencher as well as the use of a passive reference dye must be specified. The foodproof *Salmonella* Enteritidis & Typhimurium Detection LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For the PikoReal 24, Yakima Yellow (YY) has to be chosen instead of HEX.
- For users of the Agilent Mx3005P instrument: Click 'Instrument' → 'Filter Set Gain Settings' to open the Filter Set Gain Settings dialog box. For FAM, the Filter Set Gain Setting must be modified to FAM 'x2', HEX to 'x2', ROX to 'x1'.
- For CFX 96 Analysis Software: 'Apply Fluorescence Drift Correction' in 'Settings' → 'Baseline Settings' must be activated.





### 2.3.2 Preparation of the PCR Mix

Proceed as described below to prepare a 25 µL standard reaction.

Always wear gloves when handling the PCR vessels. Use material from single colonies prepared with the microproof® Suspension Buffer (Product No. KIT230178).

**Note:** The PCR strips must be stored in the provided aluminum bag with the silica gel pads to prevent liquid absorption.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or a scalpel to cut the strips apart. Tightly seal the bag afterwards and store at the recommended conditions.
2. Place the PCR tube strips 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 remove and discard the caps from the tube strips.

**Note:** To avoid unwanted liquid absorption, open strips shortly before filling. Do not leave strips open for extended periods of time.

4. Pipet samples into PCR vessels:
  - a) Pipet 20 µL of Internal Amplification Control (Product No. KIT230016) into each well.
  - b) Add 5 µL of suspended colony material to each well.

**Note:** Cells in the microproof Suspension Buffer are not inactivated before the pre-incubation step of the PCR. To reduce the risk of cross-contamination, it is recommended to prepare one PCR tube strip at a time.

5. Pipet controls into PCR vessels:
  - For the negative control, add 20 µL of Internal Amplification Control (Product No. KIT230016) into each well and add 5 µL of PCR-grade H<sub>2</sub>O (vial 2, colorless cap).
  - For the positive control, add 25 µL of foodproof *Salmonella* Enteritidis & Typhimurium Detection Control Template (vial 1, purple cap).
6. Seal the PCR vessels accurately and tightly with the colorless cap strips.
7. Mix thoroughly using a vortex centrifuge.

**Note:** Hygiena Diagnostics recommends vortex centrifuge 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.

8. 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 1000 x *g*!

9. Place the samples in your PCR cycler and run the program as described above.

**Note:** For LightCycler 480 instruments, a special adapter (Product No. MIS230006) 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.



## 2.4 Data Interpretation

The amplification of the *Salmonella* Enteritidis-specific DNA region is analyzed in the fluorescence channel suitable for FAM-labeled probes detection. The amplification of the *Salmonella* Typhimurium-specific DNA region is analyzed in the fluorescence channel suitable for the detection of HEX-labeled probes. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for ROX-labeled probes.

Compare the results from the FAM (*Salmonella* Enteritidis), HEX (*Salmonella* Typhimurium) and ROX (Internal Control) channels for each sample, and interpret the results as described in the following table:

<i>Salmonella</i> Enteritidis	<i>Salmonella</i> Typhimurium	Internal Control	Result Interpretation
FAM Channel	HEX Channel	ROX Channel	
Positive	Positive	Positive or Negative	Positive for <i>Salmonella</i> Enteritidis and <i>Salmonella</i> Typhimurium
Negative	Positive	Positive or Negative	Positive for <i>Salmonella</i> Typhimurium
Positive	Negative	Positive or Negative	Positive for <i>Salmonella</i> Enteritidis
Negative	Negative	Positive	Negative for <i>Salmonella</i> Enteritidis and <i>Salmonella</i> Typhimurium
Negative	Negative	Negative	Invalid

**Note:** A prerequisite for the unambiguous discrimination of *Salmonella* Enteritidis and *Salmonella* Typhimurium DNA as well as the Internal Control DNA in this multi-colored 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 cyclers for further information.



### 3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	<ul style="list-style-type: none"> <li>Set Channel settings to FAM, HEX or ROX.</li> </ul>
	Pipetting errors or omitted reagents.	<ul style="list-style-type: none"> <li>Check for correct pipetting scheme and reaction setup. Repeat the PCR run.</li> <li>Always run a positive control along with your samples.</li> </ul>
	No data acquisition programmed.	<ul style="list-style-type: none"> <li>Check the cycle programs.</li> </ul>
No signal increase in the ROX channel.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	<ul style="list-style-type: none"> <li>Use a recommended DNA sample preparation kit to purify template DNA.</li> <li>Dilute samples or pipet a lower amount of sample DNA (e.g., 5 µL instead of 25 µL, substituting with H<sub>2</sub>O, PCR-Grade).</li> </ul>
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> <li>Store the foodproof <i>Salmonella</i> Enteritidis &amp; Typhimurium Detection lyophilized PCR Mix at 2 to 8 °C, protected from light and moisture.</li> </ul>
	Low initial amount of target DNA.	<ul style="list-style-type: none"> <li>Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.</li> </ul>
Strong decrease of fluorescence baseline	Resuspension of lyophilized PCR mix not complete	<ul style="list-style-type: none"> <li>Always resuspend the lyophilized PCR mix thoroughly.</li> </ul>
Negative control samples are positive.	Carryover contamination is present.	<ul style="list-style-type: none"> <li>Exchange all critical solutions.</li> <li>Repeat the complete experiment with fresh aliquots of all reagents.</li> <li>Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carryover contamination.</li> <li>Add positive controls after sample and negative control reaction vessels have been sealed.</li> </ul>



Observation	Possible Reason	Recommendation
Fluorescence intensity varies.	Insufficient centrifugation of the PCR vessels. Prepared PCR mix is still in the upper part of the vessel.	<ul style="list-style-type: none"> <li>Always centrifuge reaction vessels.</li> </ul>
	Outer surface of the vessel or seal is dirty (e.g., by direct skin contact).	<ul style="list-style-type: none"> <li>Always wear gloves when handling the vessel and seal.</li> </ul>
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	<ul style="list-style-type: none"> <li>Always store the lyophilized PCR mix in the aluminum bag with the silica gel pad.</li> <li>Open PCR Strip shortly before filling.</li> </ul>

## 4. Additional Information on this Product

### 4.1 How this Product Works

The foodproof *Salmonella* Enteritidis & Typhimurium 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. The hydrolysis probe was designed to bind specifically the IC, allowing detection in the ROX channel, whereas the *Salmonella*-DNA is detected in FAM (*Salmonella* Enteritidis) and HEX (*Salmonella* Typhimurium) channels.

In cases of a negative result due to inhibition of the amplification by 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 *Salmonella* Enteritidis and *Salmonella* Typhimurium in the sample.

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

### 4.2 Test Principle

- Using the supplied sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and its associated reagents amplify and simultaneously detect fragments of specific sequences for *Salmonella* Enteritidis and *Salmonella* Typhimurium.
- 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 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 dUTP 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* 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* Enteritidis & Typhimurium Detection LyoKit, decontamination can be achieved with the provided reagents.

#### 4.4 Background Information

The genus *Salmonella*, member of the *Enterobacteriaceae* family, comprises the two species *S. enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies, of which *S. enterica* subsp. *enterica* is the most clinically significant, causing 99% of *Salmonella* infections. The subspecies are further subdivided into more than 2,000 serovars defined by somatic and flagellar antigens.

*Salmonella* Typhimurium and *Salmonella* Enteritidis belong to the subspecies *S. enterica enterica* and are the most frequently reported serovars associated with human cases of *Salmonella* infection in the EU from foodborne outbreaks. Infections with *Salmonella* Typhimurium can be caused by the monophasic or the diphasic variant. *Salmonellae* are usually transmitted to humans by eating contaminated food, which is often of animal origin. For example, human *Salmonella* Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat. [1].

Conventional microbiological methods for the detection and identification of *Salmonella* serovars are very time-consuming. The current accepted method for isolation of *Salmonella* from food and environmental primary production samples is done according to the ISO 6579, which can take up to 5 days to complete. The most widely used method to characterize *Salmonella* into its subspecies is the Kauffman-White serotyping system, based on the variability of the O, H and Vi antigens [2]. Real time PCR based methods are a fast, sensitive and specific alternative to conventional microbiology.

#### 4.5 References

1. European Food Safety Authority. (2007) The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. EFSA J. 130:2 – 352.
2. K L Hopkins, et al. (2010) Multiresistant *Salmonella enterica* serovar 4,[5],12:i:- in Europe: a new pandemic strain?. Euro Surveill. 15(22):pii=19580.

#### 4.6 Quality Control

The foodproof *Salmonella* Enteritidis & Typhimurium Detection LyoKit is function tested using the LightCycler 480 System.



## 5. Supplementary Information

### 5.1 Ordering Information

Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview and for more information, visit us at [www.hygiena.com](http://www.hygiena.com) and contact us via email or phone.

### 5.2 License Notice

The purchase price of this product includes limited, nontransferable rights under U.S. 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](mailto:outlicensing@lifetech.com).

### 5.3 Trademarks

foodproof<sup>®</sup> and microproof<sup>®</sup> are trademarks of Hygiena Diagnostics GmbH. Hygiena<sup>®</sup> is a trademark of Hygiena. Other brand or product names are trademarks of their respective holders.

### 5.4 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics, contact our Technical Support staff (for details, see [www.hygiena.com/support](http://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.

### 5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 602 34

## 6. Change Index

#### *Version 1, July 2013*

First version of the package insert.

#### *Version 2, March 2017*

License Notice changed.

#### *Version 3, March 2020*

Colony confirmation protocol added. Sample preparation method option added.

#### *Revision A, October 2023*

Rebranding and new layout. Change document tracking number.

R 602 34 20 -> INS-KIT230106-07-REVA



**Hygiena®**

Camarillo, CA 93012

USA

[diagnostics.support@hygiena.com](mailto:diagnostics.support@hygiena.com)

**Manufactured by**

**Hygiena Diagnostics GmbH**

Hermannswerder 17

14473 Potsdam

Germany

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