FOR *IN VITRO* USE ONLY

foodproof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit
– 5´Nuclease –

Version 2, November 2021

PCR kit for the qualitative detection of *Salmonella* spp., *Salmonella* Enteritidis, *Salmonella* Typhimurium using real-time PCR instruments

Order No. R 602 59-1 / R 602 59-2 / R 602 59-3

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

Store the kits at 2 to 8 °C

Table of contents

1. What this Product Does

Number of Tests

The kit is designed for 96 reactions respectively with a final reaction volume of 25 µl each. Up to 94 (single sample preparation) plus positive and negative control reactions can be analyzed per run.

Storage and Stability

- Store the kit at 2 °C 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 Contents table:

Kit Contents

Component	Label	Contents / Function / Storage
foodproof *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit R 602 59: Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bags containing a 8-tube strip mat • R 602 59-1 with white low profile tubes* • R 602 59-2 with clear regular profile tubes* • R 602 59-3 with clear low profile tubes*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for *Salmonella* spp., *S.* Enteritidis (SE) and *S.* Typhimurium (STM) 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. • For amplification and detection of *Salmonella* spp., *Salmonella* Enteritidis, *Salmonella* Typhimurium specific sequences. • Store at 2 °C to 8 °C in the aluminum bag (sealed). • **Protect from light and moisture!**
Control Template	Vial 1 (purple cap)	• R 602 59: 1 x 300 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 2 (colorless cap)	• R 602 59: 2 x 1 ml • Nuclease-free, PCR-grade H_2O. • For use as a PCR run 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: www.bc-diagnostics.com/compatibility-chart

Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and Cy5-labeled probes as well as for using low or regular profile strip tubes. In case the strip tubes don´t fit for the instrument, the samples should be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.
- DNA Extraction Kit
  - **food**proof® StarPrep Three Kit (Order No. S 400 18)[1] **or**
  - **food**proof® StarPrep Three 8-Strip Kit (Order No. S 400 18 L)[1]

  Alternatives
  - **food**proof® Magnetic Preparation Kit I (Order No. S 400 11 L)[1]
  - **micro**proof® Suspension Buffer (Order No. S 400 10)[1] only required for colony screening

- Internal Amplification Control (Order No. A 500 23)[1] only required for colony screening
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex-centrifuge Multispin MSC-6000 for PCR-strips (Order No. D 110 66)[1] **with**
- SR-32, Rotor for MSC-3000/6000 (Order No. D 110 65)[1] **or**
- Vortex-centrifuge CVP-2 for PCR-plates (Order No. D 110 67)[1]

[1] Available from BIOTECON Diagnostics; see ordering Information for details

Applicability Statement

The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit – 5'Nuclease – is intended for rapid detection of *Salmonella* spp. and identification of *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (STM) DNA from enrichment cultures of all relevant kinds of foods, feeds, environmental samples and primary production stage (PPS) samples that are potentially contaminated with *Salmonella*. The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit is intended for the food and feed industry and for food testing laboratories.

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 a FAM, HEX, ROX and Cy5 detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480 II, LightCycler® 96 (Roche Diagnostics), Mx3005P®, AriaMx (Agilent Technologies), ABI 7500® fast (Applied Biosystems) PikoReal™ 24, QuantStudio™ 5 (Thermo Scientific) and the CFX96™ Real-Time PCR Cycler (BIO-RAD).

The performance of the **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit in combination with the **food**proof® StarPrep Three Kit (Procedure A) and the **food**proof® StarPrep Three 8-Strip Kit (Procedure A) has been approved in a NordVal International method validation (certificate No. 055). For this validation study the following categories were tested: raw and ready to cook meat products, raw and ready to cook poultry products and environmental samples. For further information about the enrichment protocols please refer to ANNEX 1 at the end of the package insert.

2. How to Use this Product

2.1 Before You Begin

Precautions

Detection of *Salmonella*, Enteritidis, and Typhimurium DNA using the **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit requires DNA amplification by PCR. 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-preventive 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.

Keep the foodproof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit lyophilized PCR Mix away from light and moisture.

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 *"Additional Equipment and Reagents Required"*).

DNA Extraction

BIOTECON Diagnostics provides sample preparation kits suitable for all kind of food, feed and PPS samples (see *"Additional Equipment and Reagents Required"*).
For more product information please refer to www.bc-diagnostics.com.

Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [**food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit Control Template (vial 1, purple cap)] or use a positive sample preparation for SE and STM as positive control.

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 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.

Confirmation

If required, positive results must be confirmed by appropriate methods (e.g., reference method ISO 6579-1).

2.2 Procedure for qualitative detection of *Salmonella* from enrichment cultures

Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (SE detection), HEX (STM detection), ROX (*Salmonella* spp. detection) and Cy5 (Internal Control detection) channel. Program the PCR instrument before preparing the samples. Use the following real-time PCR-protocol for the **food**proof® *Salmonella* Genus plus 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

Note:

- For some real-time PCR-instruments the type of the probe quencher as well as the usage of a passive reference dye has to be specified. The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For LightCycler® 480 Systems I and II, color compensation is required and will be supplied by BIOTECON Diagnostics (Color Compensation Set 3; Order No. A 500 10). Please contact BIOTECON Diagnostics for further information.
- For users of the Agilent Mx3005P instrument: Click "Instrument → Filter Set Gain Settings" to open the Filter Set Gain Settings dialog box in which the gain settings may be viewed and modified. The Filter Set Gain Setting has to be modified to FAM "x2", HEX to "x2", ROX to "x1", Cy5 to "x4".
- For CFX 96™ Analysis Software: "Apply Fluorescence Drift Correction" in "Settings" → "Baseline Settings" has to be activated.

Preparation of the PCR Mix

Proceed as described below 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 strips must be stored in the provided aluminum bag with the silica gel pads to avoid liquid absorption.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or scalpel to cut the strips apart. Tightly seal the bag afterwards and store away 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. Decap the tube strips cautiously and discard the cap strips.

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

4. Pipet 25 µl sample into each PCR-vessel:

- For the samples of interest, add 25 µl sample DNA.
  (If using less volume, add PCR-grade H_2O to achieve 25 µl).
- For the negative control, add 25 µl PCR-grade H_2O (vial 3, colorless cap).
- For the positive control, add 25 µl **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit 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 vessels accurately and tightly with the colorless cap strips.

6. Mix thoroughly using a vortex centrifuge.

Note: BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-3000 (D 110 64) for PCR-strips or vortex centrifuge CVP-2 for PCR-plates (D 110 67). Dedicated protocols are available for this centrifuge.

Note: 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 g in a suitable centrifuge.

Note: If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1,000 g!

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

Note: For using any LightCycler 480 instrument, a special adapter (Order No. Z 100 24) is necessary. For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example two strips can be placed in column 1 and 12.

2.3 Procedure for colony identification of *Salmonella*

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 a FAM (SE detection), HEX (STM detection), ROX (*Salmonella* spp. detection) and Cy5 (Internal Amplification Control detection) channel. Program the PCR instrument before preparing the samples. Use the following real-time PCR-protocol for the **food**proof® *Salmonella* Genus plus 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	**30** cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds

* Fluorescence detection in step 2

Note:

- For some real-time PCR-instruments the type of the probe quencher as well as the usage of a passive reference dye has to be specified. The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit contains probes with a non-fluorescent dark quencher and no passive reference dye.
- For LightCycler® 480 Systems I and II, color compensation is required and will be supplied by BIOTECON Diagnostics (Color Compensation Set 3; Order No. A 500 10). Please contact BIOTECON Diagnostics for further information.
- For users of the Agilent Mx3005P instrument: Click "Instrument → 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 FAM "x2", HEX to "x2", ROX to "x1", Cy5 to "x4"
- For CFX 96™ Analysis Software: "Apply Fluorescence Drift Correction" in "Settings" → "Baseline Settings" has to be activated.

Preparation of the PCR Mix

Proceed as described below to prepare a 25 µl standard reaction. Always wear gloves when handling strips or caps. Use material from single colonies prepared with the **micro**proof® Suspension Buffer (Order No. S 400 10).

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

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or scalpel to cut the strips apart. Tightly seal the bag afterwards and store away 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. Decap the tube strips cautiously and discard the cap strips.

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

4. Pipetting of samples:

- Pipet 20 µl Internal Amplification Control (A 500 23) into each PCR-vessel
- Add 5 µl of suspended colony material to each well

Note: Cells in **micro**proof® Suspension Buffer are not inactivated before the pre-incubation step of PCR.

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

5. Pipetting of controls:

- For the negative control, pipet 20 µl Internal Amplification Control (A 500 23) into a PCR-vessel and add 5 µl PCR-grade H_2O (vial 3, colorless cap).
- For the positive control, add 25 µl **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit Control Template (vial 2, purple cap).

6. Seal the vessels accurately and tightly with the colorless cap strips.

7. Mix thoroughly using a vortex centrifuge.

Note: BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-6000 (D 110 66) for PCR-strips or vortex centrifuge CVP-2 for PCR-plates (D 110 67). Dedicated protocols are available for these centrifuges.

Note: 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 g in a suitable centrifuge.

Note: If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds. Avoid centrifugation forces exceeding 1,000 g!

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

Note: For using any LightCycler 480 instrument, a special adapter (Order No. Z 100 24) is necessary. For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example two strips can be placed in column 1 and 12.

2.4 Data Interpretation

The amplification of the *Salmonella*-specific DNA region is analyzed in the fluorescence channel suitable for ROX labeled probes detection. The specific DNA region for *Salmonella* serotype Enteritidis is analyzed in the fluorescence channel suitable for FAM labeled probe detection, *Salmonella* Typhimurium in fluorescence channel suitable for HEX labeled probes detection. To confirm the PCR is compliant for a negative result, an Internal Amplification Control (IC) is included in the assay. To exclude matrix-based PCR inhibition if FAM, HEX and ROX channels are negative, check the IC in Cy5 channel. A negative result is valid, if the IC has a positive signal in Cy5. Should all channels show a negative result, please refer to chapter G for troubleshooting.

Compare the results from channel FAM (Enteritidis), HEX (Typhimurium), ROX (*Salmonella* spp.) and channel Cy5 (Internal Control) for each sample, and interpret the results as described in the table below.

Channel FAM	**Channel HEX**	**Channel ROX**	**Channel Cy5**	**Result Interpretation**
Positive	Negative	Positive	Positive or Negative	Positive for *S.* Enteritidis Negative for *S.* Typhimurium Negative or Positive for another *Salmonella* spp.
Negative	Positive	Positive	Positive or Negative	Positive for *S.* Typhimurium Negative for *S.* Enteritidis Negative or Positive for another *Salmonella* spp.
Negative	Negative	Positive	Positive or Negative	Positive for *Salmonella* spp. Negative for *S.* Enteritidis and *S.* Typhimurium[1]
Positive	Positive	Positive	Positive or Negative	Positive for *S.* Enteritidis and *S.* Typhimurium Negative or Positive for another *Salmonella* spp.
Negative	Negative	Negative	Positive	Negative for *S.* Enteritidis, *S.* Typhimurium and *Salmonella* spp.
Negative	Negative	Negative	Negative	Invalid

[1]If the amplification in the ROX channel is very weak (high Cq values), the result in the FAM and/or HEX channels could be negative due to slight differences in the assays' limit of detection in this multiplex-PCR system. In this case, a prolongation of the enrichment, a second enrichment or a larger amount of sample DNA could be used to increase the sensitivity.
Due to these slight differences, a negative result in the ROX channel might also occur in the event of very weak amplification for Enteritidis and/or Typhimurium in the FAM/HEX channels.

Note: A prerequisite for the unambiguous discrimination of *Salmonella* spp., *S.* Enteritidis, *S.* Typhimurium and the Internal Control DNA in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM, HEX, ROX and Cy5. Please refer to the operation manual of your real-time PCR cycler for further information.

3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase, even with positive controls.	Incorrect detection channel has been chosen.	• Set channel settings of FAM, VIC/HEX, ROX and Cy5.
	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 program.
No signal increase in channel Cy5.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Dilute samples or pipet a lower amount of sample DNA (e.g., 20 µl PCR-grade H_2O and 5 µl sample DNA instead of 25 µl sample DNA).
	Large amounts of *Salmonella* spp., Enteritidis or Typhimurium	• If a positive signal in FAM, HEX or ROX is detected, the PCR run is valid.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the lyophilized PCR-mix **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit at 2 °C 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.	Carry-over contamination.	• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. • Add positive control after all sample and negative control reaction tubes have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR-strips. Resuspension of the PCR-mix only in the upper part of the reaction tube.	• 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 hard to dissolve.	The lyophilized PCR-mix started to rehydrate.	• Store the lyophilized PCR-mix tightly sealed in the aluminum bag with silica gel pad. • Open strips just before filling.

4. Additional Information on this Product

How this Product Works

The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit contains all necessary reagents (except for template DNA) needed for the detection of *Salmonella* spp. -, *S.* Enteritidis - and *S.* Typhimurium DNA as well as a control template for reliable interpretations of the 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 Cy5 channel, whereas the specific *S*. Enteritidis-DNA is detected in FAM, the specific *S.* Typhimurium-DNA is detected in HEX and *Salmonella* spp.-DNA is detected in the ROX channel. In case 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* in the sample. The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit minimizes contamination risk because it included a heat-labile Uracil-DNA N-Glycosylase. Primers and probes provide specific detection of *Salmonella*-, *S.* Enteritidis-, *S.* Typhimurium-DNA in food, feed and environmental samples, including samples of primary production stage (PPS). The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only.

Test Principle

1. Using the kit's sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument in combination with the supplied reagents amplify fragments of specific sequences for *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium
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 PCR's. 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* 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 **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit, decontamination can be achieved with the provided reagents.

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* subspecies I *enterica* is the most clinically significant, causing 99% of *Salmonella* infections. The subspecies are further sub-divided into more than 2,000 serovars defined by somatic and flagellar antigens. *S.* Enteritidis and *S.* Typhimurium, both belonging to the subspecies I, are the most frequently reported serovars associated with human cases of *Salmonella* infection in the EU in the year 2018 from foodborne outbreaks. *S.* Enteritidis cases are still increasing in 2018 and were most commonly associated with the consumption of contaminated eggs and egg products.
Due to their importance as human pathogens the presence of the serovars *S.* Typhimurium (mono- or diphasic variants) and *S.* Enteritidis has been tightly regulated in many countries. In the EU the regulations 2160/2003 and 200/2010 dictate how to deal with *Salmonella*. Food, feed and places of primary production have to be tested regularly for relevant *Salmonella*e to prevent human *Salmonella* infections transmitted through contaminated food. The current accepted microbiological method for isolation and detection of *Salmonella* according to the ISO 6579 in combination with the Kauffman-White serotyping system, taking up to 5 days to complete. Because this method is very time consuming, PCR has been introduced to the food, feed and primary production industry as a highly sensitive, specific and fast alternative detection method [1, 2, 3, 4].

References

1. European Food Safety Authority. The European Union summary report on trends and sources of zoonoses, zoonotic agents resistance and foodborne outbreaks in 2017. 10.2903/j.EFSA J. 2018.5500

2. Mehnert WH *et al* (2000) Deutscher Zoonosen-Trendbericht 2000. BgVV.

3. Scheu PM *et al* (1998) Detection of pathogenic and spoilage micro-organisms in food with the polymerase chain reaction. *Food Microbiology* **15**, 13-31.

4. Scheu P *et al* (1998) Evaluation of a PCR-ELISA for food testing: Detection of selected Salmonella serovars in confectionery products. *Food Biotechnology* **12**, 1-12.

Quality Control

The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit is function tested using the LightCycler® 480 II System.

5. Supplementary Information

5.1 Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

5.2 License

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.

5.3 Trademarks

foodproof® is a trademark of BIOTECON Diagnostics GmbH.
Other brand or product names are trademarks of their respective holders.

5.4 Contact and Support

If you have questions about this or any other product of BIOTECON Diagnostics, please contact our Technical Support staff (for details see www.bc-diagnostics.com). Our scientists commit themselves to providing rapid and effective help. We also want you to 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, June 2020
First version of the package insert.

Version 2, November 2021
Addition of NordVal International logo.
Additional information in the Applicability Statement.
Addition of ANNEX 1: NordVal #055 Validation Table.

ANNEX 1: NordVal #055 Validation Table

for the foodproof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit

The following table shows the recommended enrichment time for the tested food categories and environmental samples in Buffered Peptone Water (BPW) in combination with **food**proof® StarPrep Three Kit (order No. S 400 18) and **food**proof® StarPrep Three 8-Strip Kit (order No. S 400 18 L) that have been analyzed for the NordVal International method validation of the **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit (certificate No. 055).

For further information regarding the DNA extraction procedures below, please refer to the appropriate BIOTECON Diagnostics package inserts on: www.bc-diagnostics.com.

DNA Extraction	Enrichment time in BPW at 37°C ± 1°C	DNA extract for PCR
foodproof® StarPrep Three Kit Extraction Procedure A STANDARD	18 h ± 2 h	25 µl
foodproof® StarPrep Three 8-Strip Kit Extraction Procedure A STANDARD	18 h ± 2 h	25 µl

Tested food categories:

- raw and ready to cook meat products
- raw and ready to cook poultry products
- environmental samples

Sample size: 25 g / 225 ml BPW

Reference method: ISO 6579-1 (2017) and ISO TR 6579-3 (2014)

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