Salmonella Genus DNA Detection Test Kit

vetproof® *Salmonella* qPCR LyoKit

Revision B, April 2024

Real-time PCR kit for the qualitative detection of *Salmonella* spp. in primary production samples.

Product No. KIT230197/KIT230198

96 reactions

MA No. FLI-C 055. German instructions for use are registered according to German legislation. (§ 11 Absatz 2 TierGesG)

Store at 2 to 8°C

For veterinary use only
For *in vitro* use only

Table of Contents

1. Product Description

The vetproof® Salmonella qPCR LyoKit is a real-time PCR Kit for the qualitative detection of *Salmonella* spp. in primary production samples (e.g., boot swabs, faeces and dust samples from chicken production). The kit contains all reagents and controls required for the detection of *Salmonella* spp. Primers and probes are used for the specific detection of *Salmonella* DNA in veterinary and primary production samples. The Internal Control (IC, VIC channel) is included in the assay to rule out PCR inhibition, preventing false-negative results. A negative signal in FAM (*Salmonella* spp.) with a positive IC shows that the samples are negative for *Salmonella* spp.

The vetproof® Salmonella qPCR LyoKit was validated according to DIN EN ISO 16140-2:2016 in an external expert lab. The certificate (No. 2011LR42) can be downloaded at www.microval.org or here.

1.1 Number of Tests

The kit is designed for 96 reactions with a final reaction volume of 25 µL each.

1.2 Storage and Stability

Store the kit at 2 to 8 °C through the expiration date printed on the label. Store the 8-tube PCR strips with the lyophilized reagents in the aluminum bags to protect them from light and moisture. Close the bag tightly after each use.

1.3 Kit Contents

	Component	Format	Function
1	PCR Plate, separable into 8-tube strips, prefilled with 96 reactions (lyophilized reaction mix)	• Aluminum bag containing 96 well plate with 8-tube PCR strips • KIT230197 (LP) with "low profile" 8-tube PCR strips* • KIT230198 (RP) with "regular profile" 8-tube PCR strips*	• Ready-to-use PCR-reaction-mix, containing primers and hydrolysis probes, Internal Control, Taq-DNA-Polymerase and Uracil-DNA-Glycosylase (UNG, heat-labile) ** • For the amplification and detection of • *Salmonella*-specific sequences • Protect from light and moisture • 25 µL per reaction.
2	Control Template	• One (1) vial with purple cap	• 1 x 900 µL • Contains a stabilized solution of DNA • For use as a PCR run positive control
3	H_2O PCR-grade	• Two (2) vials with clear caps	• 2 x 1 mL nuclease-free, PCR-grade H_2O • For use as a PCR run negative control
4	Cap Strips	• Plastic bag containing 8-cap strips	• 12 x 8-cap strips • For sealing the PCR strips after the addition of samples

***** Compatibility of PCR tubes with real-time PCR instruments must be checked prior to assay set-up.
** The PCR reaction mix contains Taq polymerase for PCR and Uracil-DNA N-Glycosylase (UNG) for efficient degradation of previously amplified DNA. The real-time PCR kit contains dUTP (deoxyuridine triphosphate) instead of dTTP. This technique relies on the incorporation of dUTP during all amplification reactions. The UNG cleaves DNA at any site where a deoxyuridine residue has been incorporated. The resulting abasic sites are hydroly zed due to the high temperatures during the initial denaturation step and can no longer serve as PCR templates.

1.4 Applicability Statement

This kit is compatible with all real-time PCR instruments suitable for detection of FAM and VIC fluorophores.

1.5 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for the detection of FAM and VIC-labeled probes
- DNA extraction method (see: *Preparation of Samples > DNA extraction*)
- Centrifuge for 8-strip PCR tubes (150 x g)
- Pipettes
- Nuclease-free disposable filter-tips
- Disposable powder-free gloves
- Scissors

1.6 Preparation of Samples

Sample material and enrichment

Relevant sample material (such as boot swabs, dust, or fecal samples) has to be enriched in the given volume ratio in buffered peptone water (BPW) for 18 ± 2 hours at 37 ± 1 °C according to ISO 6579-1:2017. Enrichment in Stomacher® bags is recommended.

For feces and sample material with a high content of soil, Hygiena Diagnostics suggests a selective sub-cultivation with pre-warmed Mossel broth (1 ml of first enrichment in 9 ml Mossel broth with incubation for at least 5 ± 0.5 hours at 37 ± 1 °C and 150 revolutions per minute).

DNA extraction

For DNA extraction, foodproof® StarPrep® Three (Product no. KIT230187) or foodproof® StarPrep One Kit (Product nos. KIT230175, or KIT230176 for use with 8-Channel-Pipettes) are recommended.

2. How to Use This Product

2.1 Good Laboratory Practices for PCR

- Assays must be performed by qualified laboratory personnel only.
- Wear disposable powder-free gloves at any stage of running the assay and/or sample preparation. Change gloves when changing work areas or if you suspect that they are contaminated.
- Treat all biological materials as potentially biohazardous, including all field samples.
- Avoid prolonged exposure of the lyophilized reaction mix to direct light and moisture.
- Use nuclease-free lab ware (e.g., pipettes, pipette tips, reaction vials).
- To avoid cross-contamination of samples and reagents, use aerosol-preventive pipette tips.
- Strict adherence to the test protocol will lead to achieving the best results.
- Physically separate the workplaces for DNA/RNA extraction, PCR setup (work area 2) and PCR amplification. (work area 3) to minimize the risk of carry-over contamination.
- Use a PCR hood for all pipetting steps. As the kit is provided as a ready-to-use lyophilized mix, a dedicated work area for reagent setup (work area 1) is not required.
- Never move any materials from work area 3 to work area 2 or from work area 2 and 3 to work area 1.
- Decontaminate PCR laboratories with bleach or alternative DNA decontaminant and UV light (optional) after testing.

2.2 Procedure

Real-time PCR Protocol

The following procedure is optimized for a real-time PCR instrument with a FAM (*Salmonella*) and a VIC (Internal Control) detection channel.
Program the real-time PCR instrument before preparing the samples. Use the real-time PCR protocol below for the vetproof Salmonella qPCR LyoKit. For details on how to program the experimental protocol, see the instrument operator's manual for the real-time PCR cycler used:

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 real-time PCR instruments without a VIC detection channel, HEX can be used.
- 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 vetproof Salmonella qPCR LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.

2.2.2 Preparation of the PCR Mix

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

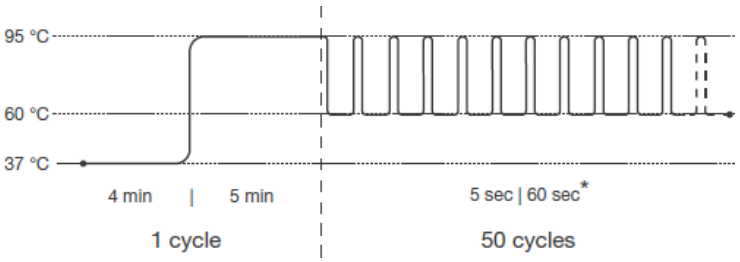
Always wear gloves when handling the PCR tubes. Sample material should be suitable for PCR concerning purity, concentration and presence of inhibiting substances.

Note: The lyophilized reaction mix is only stable if the PCR strips are stored sealed in the provided aluminum bag with the silica gel pads to avoid liquid absorption.

1. Remove the needed number of 8-strip PCR tubes from the aluminum bag. Use scissors to cut the required amount of reaction tubes. Tightly seal the bag and make sure the silica gel is included.
2. Place the 8-strip PCR tubes 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 clear cap strips.
    a. **Note:** Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips just before filling.
4. Pipette 25 µL sample into each PCR tube and resuspend the pellet by cautiously pipetting up and down.
    a. Add 25 µL sample DNA to the lyophilized reaction mix. If less sample volume is available, add PCR-grade H_2O to a total volume of 25 µL.
    b. For the negative control, add 25 µL H_2O PCR-grade (vial with clear cap).
    c. For the positive control, add 25 µL of Control Template (vial with purple cap).
5. Seal the tubes tightly with new cap strips.
    a. **Note:** Alternatively, resuspend the pellet after closing the tubes by mixing thoroughly. To reduce the risk of cross-contamination, only one PCR strip should be processed at a time.
    b. When using RP PCR strips, make sure the strips are sealed tightly when mixing.
6. Briefly (5 seconds) spin the PCR strips in a suitable centrifuge (150 x g).
7. Put the samples in the real-time PCR cycler and start the program.
    a. **Note:** For some PCR instruments the PCR strips should be placed evenly distributed into the cycler block (e.g., place two strips in columns 1 and 12).

2.2.3 Workflow

Program set-up: Set up the PCR instrument before preparing the samples. Assign these channels:
FAM (Salmonella) and VIC (Internal Control)



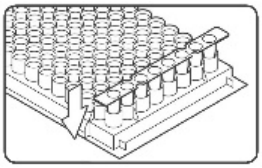
Pre-incubation: 1 Cycle
Step 1: 37 °C for 4 minutes
Step 2: 95 °C for 5 minutes
Amplification: 50 Cycles
Step 1: 95 °C for 5 seconds
Step 2*: 60 °C for 60 seconds
* Measurement in Step 2

2.2.4 Implementation: Real-time PCR

Take appropriate precautions to prevent contamination, e.g. by using filter tips and wearing gloves.

1. PLACE STRIPS IN RACK

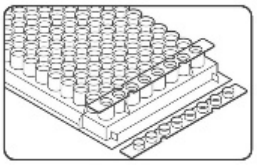
Take needed number of PCR tubes out of the aluminum bag. Important: close the bag tightly afterwards. Place strips in a suitable PCR tube rack. If needed, gently tab the tubes to move the lyophilized pellets to the bottom of all tubes.



2. OPEN PCR STRIPS

Open strips carefully just before filling and discard caps.

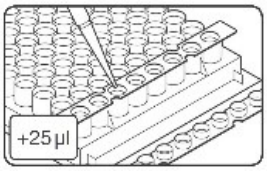
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

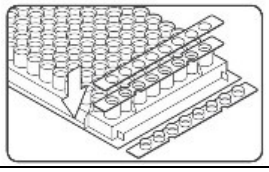
Pipet 25 µL of samples, negative control (clear caps) or Control Template (purple cap) into respective wells.

If using a lower sample volume, add PCR-grade H_2O to a total volume of 25 µL.



4. SEAL

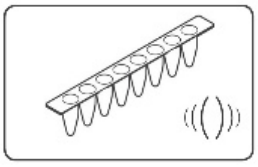
Seal the tubes with the 8-cap strips accurately.



5. MIX AND CENTRIFUGE

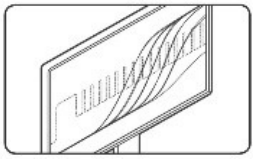
Resuspend the pellet by mixing thoroughly after sealing. Alternatively resuspend the pellet by repeatedly pipetting up and down in Step 3.

Spin strips for 5 seconds at approx. 150 x g in a suitable centrifuge.



6. START REAL-TIME PCR RUN

Cycle samples as described above. Evenly spread the strips in the cycler block (e.g., two strips can be placed in columns 1 and 12).



2.1 Validation and Interpretation

The amplification of *Salmonella* DNA is analyzed in FAM, the Internal Control is analyzed in the VIC detection channel. To verify true negative results and exclude PCR inhibition as a cause of a negative signal in FAM, check for amplification for the internal control in the VIC channel.

Interpretation of Results

Channel FAM	Channel VIC	Results
Positive (Ct value: 10 – 50)	Positive or negative	Positive for *Salmonella* spp.
Negative	Positive (Ct value: 25 – 40)	Negative for *Salmonella* spp.
Negative	Negative	Invalid

Positive Result: Detection of *Salmonella* spp. (field strain or vaccine strain) in the sample. Regulatory requirements vary by country, further analysis to confirm and to determine serotype and to differentiate field and vaccination strains may be required.

Figure 1 shows an example of an amplification curve for samples with high and low concentrations of *Salmonella* DNA. Also shown is the curve of a sample containing no *Salmonella* DNA. For this sample, only the internal control gives a positive signal curve (Figure 2), presenting a true negative result for *Salmonella* spp.

Note: Suitable calibration of FAM and VIC channels in your real-time PCR instrument is required for the discrimination of *Salmonella* and the Internal Control. Follow the instructions for your real-time PCR cycler.

Figure 1

FAM-Channel (*Salmonella* detection)

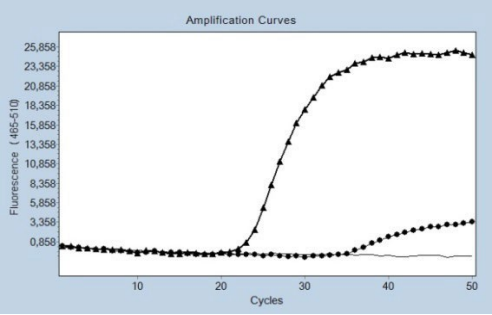
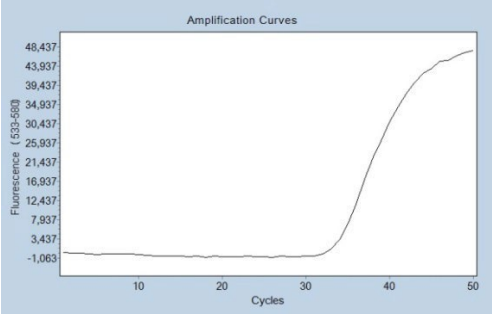


Figure 2

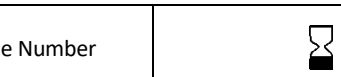
VIC-Channel (internal control)



Legend:
Triangle: high concentration of *Salmonella* DNA
Circle: low concentration of *Salmonella* DNA
Thin Line: sample without *Salmonella* DNA

3. Supplementary Information

Glossary

REF	Product Reference Number		Expiry (Expiration) Date
	Kit Size/Reactions		Protect from Moisture
	Store at		Protect from Heat and Direct Sunlight
	Batch		Manufacturer

Quality Control

All products are monitored by our quality control on a batch-to-batch basis.

Trademarks

vetproof ®, **food**proof ® and StarPrep® are registered trademarks of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

Warranty and Disclaimer of Liability

"Limited Warranty" and "Disclaimer of Liability": Hygiena Diagnostics GmbH warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

(1) The product is used according to the guidelines and instructions set forth in the product literature;

(2) Hygiena Diagnostics GmbH does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by Hygiena Diagnostics GmbH; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;

(3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;

(4) Hygiena Diagnostics GmbH does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of Hygiena Diagnostics GmbH;

(5) Hygiena Diagnostics GmbH does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;

(6) Hygiena Diagnostics GmbH reserves the right to replace or allow credit for any modules returned under this warranty.

Regulatory Disclaimers

For veterinary use only. For *in vitro* use only.

Regulatory requirements vary by country; this product may not be available in your geographic area. For information on availability, please contact Hygiena Diagnostics GmbH.

4. Revision Index

Version 1: Pre-launch version.
Version 2: Final version following consultations with German registration body.
Version 3: Added a note about validation, according to DIN EN 16140-2:2016.
Revision A: Changed kit name, product code, layout product instructions and supplier info.
Revision B: Final version after consultations with the German registration office. Minor text edits after translation to American English.

5. Supplier Information

Manufactured by
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