vetproof® *Salmonella* Detection Kit
- 5'Nuclease -

Order No. V 900 27

Instructions for use

Version 3, February 2020 – EN

For in vitro veterinary diagnostics only (Chicken)

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

▽96 Reactions

Real-time PCR Kit for the qualitative detection of *Salmonella* spp. in primary production samples (e.g. sock swabs, feces 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 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 **vet**proof® *Salmonella* Detection Kit 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 www.bc-diagnostics.com.

A. Kit contents

Component	Format	Function
PCR Plate, with 96 reactions (lyophilized reaction mix) 1	Aluminum bag containing 8-tube PCR-strips • V 900 27-1 (LP) with „low profile" 8-tube PCR-strips * • V 900 27-2 (RP) with „regular profile" 8-tube PCR-strips *	• Ready-to-use PCR-reaction-mix, containing primer 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.
Control Template 2	Vial 1 (purple cap)	• 1 x 900 µl • Contains a stabilized solution of DNA • For use as a PCR run positive control
H_2O PCR-grade 3	Vial 2 (colorless cap)	• 2 x 1 ml • Nuclease-free, "PCR-grade" H_2O • for use as a PCR run negative control
Cap Strips 4	Plastic bag containing 8-cap strips	• 12 x 8-cap strips to seal the reaction tubes

* Compatibility of PCR-tubes with real-time PCR-instruments is provided by BIOTECON Diagnostics online: www.bc-diagnostics.com/compatibility-chart

** 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 instead of dTTP: This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions. 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.

Store the kit at 2 °C 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 and protect them from light and moisture. Close the bag carefully after use.

Additional equipment required

- Real-time PCR-cycler suitable for detection of FAM and VIC labeled probes
- Nuclease-free pipette tips
- Pipettes
- Centrifuge for 8-strip PCR-tubes (150 x g)

B. Sample preparation

Sample material and enrichment

Relevant sample material such as sock 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 samples material with a high content of soil, BIOTECON Diagnostics suggest a selective sub cultivation with pre-warmed Mossel broth (1 ml of first enrichment in 9 ml Mossel broth for at least 5 ± 0.5 h at 37 ± 1°C and 150 revolutions per minute).

DNA extraction

For DNA extraction, **food**proof® StarPrep Three (order number S 400 18) or **food**proof® StarPrep One Kit (order number S 400 07, or S 400 14 L for use with 8-Channel-Pipettes) are recommended. For simultaneous detection and differentiation of vaccine strains (SE Vaccine Detection Kit 1, order number V 900 01) from one extraction, **food**proof® StarPrep Three should be used.

For details see ordering information from BIOTECON Diagnostics.

C. 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 **vet**proof® *Salmonella* Detection Kit. 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 real-time PCR instruments without VIC detection channel, HEX can be used. For PikoReal®, Yakima Yellow (YY) needs to 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 **vet**proof® *Salmonella* Detection Kit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.

D. 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 (see chapter B).

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

1. Take the needed number of 8-strip PCR tubes out of the aluminum bag. Use scissors to cut the required amount of reaction wells. 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. Uncap the tube strips cautiously and discard the clear cap strips.

   Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips just before filling.
4. Pipet 25 µl sample into each PCR tube and resuspend the pellet by cautiously pipetting up and down.
   - 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.
   - For the negative control, add 25 µl H_2O PCR-grade (vial 2, colorless cap).
   - For the positive control, add 25 µl of Control Template (vial 1, purple cap).

5. Seal the vessels tightly with new cap strips (colorless).

   Note: Alternatively, resuspend the pellet after shutting the vessels by mixing thoroughly. To reduce the risk of cross-contamination, only one PCR-strip should be processed at a time.

   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 (see chapter C).

   Note: For some PCR-instruments the PCR-strips should be placed evenly distributed into the cycler block (e.g. place two strips in column 1 and 12).

E. Data interpretation

The amplification of *Salmonella* DNA is analyzed in FAM, internal control is analyzed in 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 the results for both channels:

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

Figure 1 shows examples for amplification curves of samples with high and low concentration 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.

Figure 1

FAM-Channel (*Salmonella* detection)

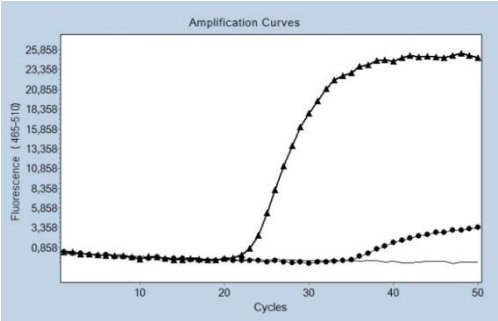
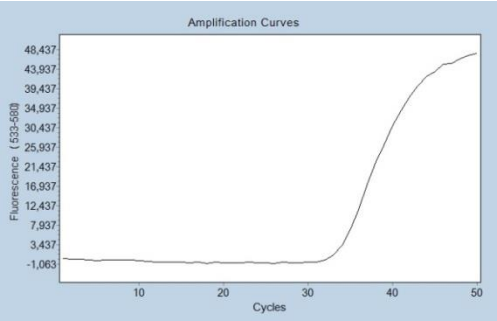


Figure 2

VIC-Channel (internal control)



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

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

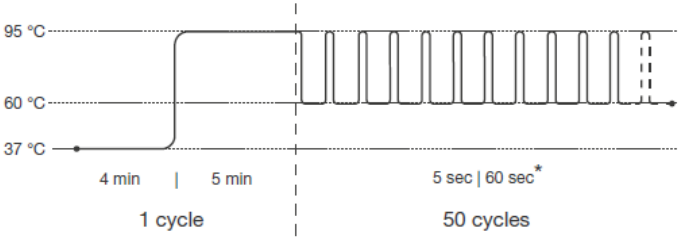
Positive Result: Detection of *Salmonella* spp. (field strain or vaccination strain) in the sample. Regulatory requirements vary by country, further analysis to determine serotype and differentiate field and vaccination strains may be required.

F. Protocol and workflow

Program setup

Set up the PCR-instrument program before preparing the samples. Assign these channels:

- FAM (*Salmonella*) and VIC (internal control).



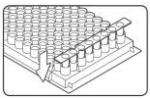
Pre-incubation: 1 Cycle
Step 1: 37 °C for 4 minutes
Step 2: 95 °C for 5 minutes
Amplification: 50 Cycles
Step 1: 95 °C for 5 seconds
Step 2*: 60 °C for 60 seconds
* Measurement in Step 2

Implementation: real-time PCR

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

1. PLACE STRIPS IN RACK

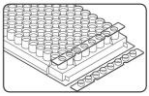
Take needed number of PCR tubes out of the aluminum bag. Important: close the bag tightly afterwards. Place strips in a suitable PCR tube rack. If needed, gently tab the tubes to move the lyophilized pellets to the bottom of all tubes.



2. OPEN PCR-STRIPS

Open strips carefully just before filling and discard caps.

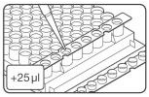
Do not leave open longer than necessary.



3. ADD SAMPLES AND CONTROLS

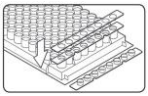
Pipet 25 µl of samples, negative control (colorless caps) or Control Template (purple cap) into respective wells.

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



4. SEAL

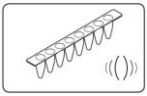
Seal the tubes with the 8-Cap strips accurately.



5. MIX AND CENTRIFUGE

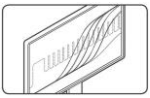
Resuspend the pellet by mixing thoroughly after sealing. Alternatively resuspend the pellet by repeatedly pipetting up and down in step 3.

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 column 1 and 12).



G. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase, even with positive controls.	Incorrect detection channel has been chosen.	• Set channel settings of FAM and VIC.
	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 cycler program.
No signal increase in channel in VIC/HEX.	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).
	High amounts of *Salmonella* DNA.	• If a positive signal in FAM is detected, the PCR run is valid.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the lyophilized **vet**proof® Kit 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 caps.
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.

H. Symbols

REF	Product reference number		Expiry
	Kit size / reactions		Protect from moisture
	Store at		Protect from heat and direct sunlight
	Batch		Manufacturer

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 bcd@bc-diagnostics.com

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 DIN EN 16140-2:2016

BIOTECON Diagnostics GmbH
Hermannswerder 17
14473 Potsdam – Germany
Telefon +49 (0) 331 2300-200
Fax +49 (0) 331 2300-299
bcd@bc-diagnostics.com
www.bc-diagnostics.com

V 900 27 23