Salmonella Species-Enteritidis-Typhimurium DNA Test Kit

vetproof® Salmonella Species-Enteritidis-Typhimurium qPCR Kit

Revision A, March 2024

Real-time PCR kit for the simultaneous detection of *Salmonella* spp., *S*. Enteritidis and *S*. Typhimurium DNA in avian and swine samples.

Product No. KIT230355

Reagents for 100 reactions

Store at -20°C

For veterinary use only
For *in vitro* use only

Table of Contents

1. Product Description

The vetproof® Salmonella Species-Enteritidis-Typhimurium qPCR Kit (Spp-Se-St qPCR) will detect the presence of DNA from *Salmonella* spp. (Spp) and/or *Salmonella* Enteritidis (Se) and/or *Salmonella* Typhimurium (St) in extracts from avian or swine derived samples. Primers and probes are specific for *Samonella* spp. and *S*. Enteritidis and *S*. Typhimurium; each probe is labelled with a specific fluorophore which is detected in a designated channel on the qPCR thermocycler. After extraction of nucleic acids, samples are added to wells along with the dedicated reaction mix. The prepared wells are placed in the real-time PCR cycler for amplification and detection.

The Spp-Se-St qPCR assay enables simultenous detection of:

- *Salmonella* species (Spp; detected in FAM channel)
- *Salmonella* Enteritidis (Se; detected in Texas Red channel)
- *Salmonella* Typhimurium (St; detected in CY-5 channel)
- Internal Control (IC; detected in HEX channel)

1.1 Number of Tests

The kit contains reagents for 100 PCR reactions with a final reaction volume of 25 µl each.

1.2 Storage and Stability

Upon receipt, store reagents at - 20°C.

1.3 Kit Contents

The vetproof® Salmonella Species-Enteritidis-Typhimurium qPCR Kit contains the following reagents:

1. **Master Mix**, 2 vials, liquid (2 x 675 µl) (yellow cap)
2. **Primer/Probe Mix & Internal Control (PP/IC)**, 2 vials, liquid (2 x 415 µl) (white cap)
3. **Control Template** (Positive Control), 1 vial, liquid (1 x 200 µl) (purple cap)
4. **H_2O PCR-grade** (Negative Control), 1 vial, molecular grade water, liquid (1 x 1000 µl) (clear cap)

Note: Control Template (Positive Control): Diluted Spp/Se/St plasmid with cloned target sequence standardized to represent significant amounts of the target.

1.4 Applicability Statement

This kit is compatible with all real-time PCR instruments suitable for the detection of FAM,Texas Red, CY-5 and HEX fluorophores.

1.5 Additional Equipment and Reagents Required

- DNA extraction method (see: Preparation of Samples > DNA extraction)
- Enrichment media
- Real-time PCR cycler suitable for the detection of FAM, Texas Red, CY-5 and HEX-labeled probes
- Vortex mixer (x2)
- Mini-centrifuge (x2)
- Heating block
- PCR plate-spinner
- Single, 8 or 12 channel pipettes
- Nuclease-free disposable filter-tips for volumes of 1-1000 µl
- Plates, strips (+caps) or microtubes for DNA extraction
- Nuclease-free tubes for preparation of reaction mix
- Plates for PCR reaction (suitable for use with your real-time PCR cycler)
- Heat resistant seales for plate
- Disposable powder-free gloves
- Refrigerator and freezer

1.6 Preparation of Samples

Sample material and enrichment
Avian and swine derived samples.

For sample enrichment protocols, it is recommended to follow the requirements as described in:
- ISO 6579
- Bacteriological Analysis Manual, Salmonella Chapter
- National Program Imporvement Plan Program Standards

DNA extraction
Before running the PCR, DNA must be extracted from the sample.
The extraction method chosen to be used with the vetproof® Salmonella Species-Entertidis-Typhimurium qPCR Kit must be suitable for DNA extraction. Recommended are lysis buffer (e.g. vetproof® Lysis Buffer A (Product no. KIT230344)), spin column extraction methods or magnetic bead extraction methods (e.g. vetproof® MagBead Extraction Kit I (Product no. KIT230342)).
Extracted DNA can be stored at -20°C prior to running the PCR.
For the matrices to be examined, it is advised to validate chosen extraction method internally prior to generating results.

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.
- Handle all reagents with care.
- Treat all biological materials as potentially biohazardous, including all field samples.
- Never pipette anything by mouth. There must be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
- 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.
- Dedicate one airspace for kit storage/reagent preparation (Room 1, Clean Room) and another airspaece (Room 2) for running the assay and sample preparation/extraction (Room 2). A third airspace is optional (Room 3) for dedicated amplification/running the assay.
- Never move any materials and equipment from Room 2 or 3 to Room 1.
- Decontaminate PCR laboratories with bleach or alternative nucleic acid decontaminant and UV light (optional) after testing.
- Always used a positive control (Control Template) and negative control (H_2O PCR-grade) for every PCR run.

2.2 Procedure

Recommended workflow protocol
When running complete assay including DNA extraction in 1 day
1. Start in Room 1 with reagent preparation.
2. Go to Room 2/3 for DNA extraction and running assay.
3. Never go from Room 2/3 to Room 1 during the same day.

When doing DNA extraction first
Day 1:
1. Start in Room 2 with DNA extraction.

Day 2:
1. Start in Room 1 with reagent preparation.
2. Go to Room 2/3 for running the assay.
3. Never go from Room 2/3 to Room 1 during the same day.

Reagent preparation (Room 1 – Clean Room)
1. Defrost reagents at room temperature.
2. Vortex reagents thoroughly and briefly spon to remove any residues from the lid.
3. Calculate total volumes of Master Mix and PP/IC required for all reactions (Reaction Mix). Do not forget to include reactions for controls (minimum one positive and one negative), and to compensate for dead volume (+1 reaction for instance).

Reaction Mix (20 µl)	
Master Mix	12.5 µl
PP/IC	7.5 µl

4. Put the total volume of required Master Mix into a clean microtube.
5. Add the total volume of required PP/IC to to the microtube.
6. Vortex microtube to mix thoroughly, and briefly spin to remove any residues from the lid.

Assay preparation (Room 1)
1. Take a suitable qPCR plate and record location of samples on template.
2. Add 20 µl of Rection Mix as prepared above for every sample plus controls. The plate does not need to be cooled.
3. Add 5 µl of H_2O PCR-grade (negative control) into control well. This is a reagent and environment control (Optional Control for Room 1).
4. Cover plate and take into Room 2.

DNA amplification (Room 2/3 – Extraction/Amplification Room)
1. Add 5 µl of Control Template (Positive Control) into control well.
2. Add 5 µl of H_2O PCR-grade (Negative Control) into control well. This is an environment control.
3. Add 5 µl of sample (DNA extract) into each sample well.
4. Cover plate with heat resistant sealer.
5. Spin plate for 30-60 seconds at 200-1000 x g.
6. Place plate in qPCR thermocycler and run using the specified thermal cycler prgran in the table (qPCR program at normal ramp speed).

Temperature	Time	No. of Cycles
95°C	3 min	1
95 °C	15 sec	40
60 °C	60 sec	
Data collection (@ 60 °C): FAM = Salmonella Species Texas Red = Salmonella Enteritidis CY-5 = Salmonella Typhimurium HEX = Internal Control		

Note: Do not use fast mode.

Note: Alternative channel names for reporter dyes:

- FAM: no alternative name
- Texas Red: no alternative name
- CY-5: Quasar 670
- HEX: VIC, Yakima Yellow, CAL Fluor Orange 560, Alexa 532

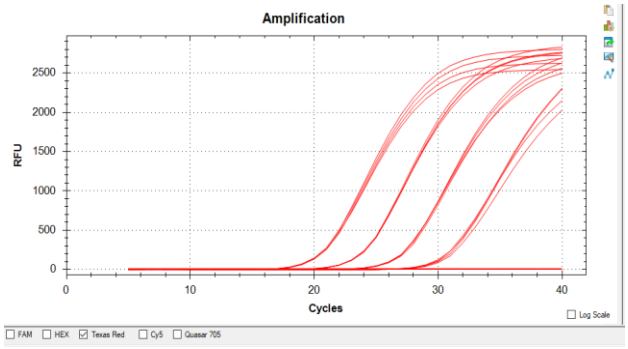
2.3 Validation and Interpretation

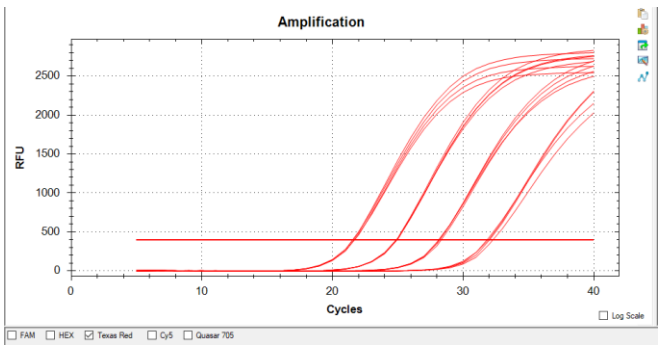
Analysis settings

Bio-Rad CFX96™	Applied Biosystems® 7500	Stratagene Mx3005P™
Fluorescence drift correction: Yes	Passive reference : no	Amplification-based threshold
Cycles to analyze: 5 – 40	Baseline cycles: 3 – 15	Adaptive baseline

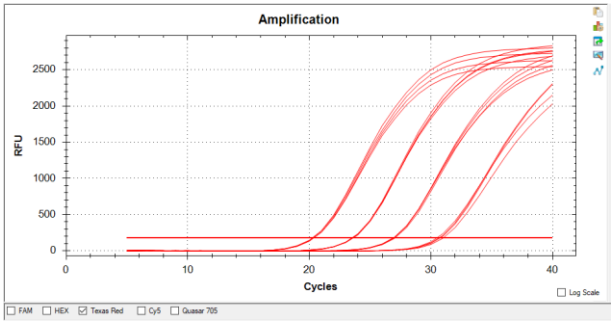
Note: Other PCR machines can be used. Contact BioChek for further information regarding suitable qPCR thermocyclers.

Setting thresholds in the PCR cycler software
Go to the part of the sogtware where you can see the amplification curves.
Select all wells on the plate, select linear view and select the FAM channel. Turn off other channels.





Depending on the amount of samples the linear curves should look like the ones in the picture above on the left. To set the threshold look at which cycle the first curve starts to form, in this case around cycle no. 20.
Look at which cycle the first formed curve goes up in a straight line, in this case around cycle 24. See picture above on the right.
The threshold is placed for demonstration purposes at the point where the curve becomes a straight line. The threshold should be set halfway between the fluorescence of cycles 20 and 24. See picture below.



Repeat this process for theTexas Red, CY-5 and HEX channel.

Note: After setting the thresholds for all channels, keep using those thresholds for all future PCR runs.

Validation of the PCR run
The following must apply for the PCR run to be valid:

	Spp (FAM) Cq values	Se (Texas Red) Cq values	St (CY-5) Cq values	IC (HEX) Cq values	Interpretation
Positive Control (Control Template)	22-33	22-33	22-33	Not considered	Valid Control
Negative Control (H_2O PCR-grade)	N/A*	N/A*	N/A*	26-34	Valid Control

*No Cq value

Note: Repeat the PCR plate in event of control failure.

Validation and interpretation of sample results
Always check validity of the amplification curves.

Spp (FAM) Cq values	Se (Texas Red) Cq values	St (CY-5) Cq values	IC (HEX) Cq values	Interpretation
<40	<40	<40	Not considered	Positive Sample Spp and/or Se and/or St
N/A*	N/A*	N/A*	26-34	Negative sample
N/A*	N/A*	N/A*	N/A* or <26 / >34	Invalid well**

*No Cq value
**The assay is invalid for the particular sample and should be repeated with a 1/10 dilution of a new made DNA extract of the sample.

Note: For sample results with a Cq between 38 and 40, it is recommended to check the amplification curve. If the curve is good, report the sample as positive. Otherwise repeat the sample.

Note: For final diagnosis qPCR positives should be considered presumptive and confirmed by standard reference methods or alternative tests for *Salmonella*.

3. Supplementary Information

Symbols 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. A certificate of analysis (CoA) is available from BioChek.

Ordering Information

This kit and associated products are available from BioChek. For a complete overview and for more information, please visit our website at www.biochek.com.

Trademarks

vetproof® is a trademark of Hygiena Diagnostics GmbH.

Warranty and Disclaimer of Liability

"Limited Warranty" and "Disclaimer of Liability": BioChek B.V. 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. BioChek B.V. does not warrant its product against any and all defects when: the defect is a result of material or workmanship not provided by BioChek B.V.; 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. BioChek B.V. 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 BioChek B.V.;
5. BioChek B.V. 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. BioChek B.V. 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 BioChek.

4. Revision Index

Version 1: New document.

Revision A: Changed kit name, product code, kit content (reagents: name, color of caps, number of vials and volumes), layout product instructions and supplier information.

5. Supplier Information

AUTHORIZED REPRESENTATIVE

BioChek B.V.
Fokkerstraat 14
2811ER Reeuwijk - Netherlands
Phone +31 (0) 331 2300-200
Website: www.biochek.com
E-mail: info@biochek.com

Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam - Germany