Salmonella Species DNA Test Kit

BioChek qPCR assays

For feed and food applications

Catalogue Number: MP104

Description of test

The Salmonella Species DNA Test Kit (Salmonella qPCR) will detect the presence of DNA from *Salmonella* Species (Salmonella) in extracts from the following samples:

- food products (Ready-to-eat, Ready-to-reheat, meat products, ingredients and specific foods)
- feed products (low moisture and high moisture products)
- primary production samples (PPS: animal faeces, boot socks, dust samples, litters etc.)
- production environmental samples (dusts and residues, swabs, cleaning and process water etc.)

Primers and probes are specific for Salmonella; the probe is labelled with a specific fluorophore which is detected in a designated channel on the qPCR thermocycler. After extraction of the nucleic acids, samples are added to plates along with the dedicated reaction mix. The prepared wells are placed in the qPCR cycler for amplification and detection.

The Salmonella qPCR test kit enables the simultaneous detection of:

- Salmonella (detected in FAM channel)
- Internal Control (IC; detected in HEX channel)

Reagents and materials provided

Salmonella qPCR (catalogue number MP104) contains reagents for 100 25 µl PCR reactions.

1. **Salmonella Primer/Probe mix with Internal Control (PP/IC),** 2 vials (yellow cap), liquid (412.5 µl)
2. **DNA Mastermix I,** 2 vials (black cap), liquid (675 µl)
3. **qPCR Negative Control,** 4 vials (blue cap), molecular grade water (60 µl)
4. **Salmonella qPCR Positive Control,** 2 vials (red cap), diluted Salmonella plasmid with cloned target sequence standardized to represent significant amounts of the target (60 µl)

Storage conditions: Upon receipt, store qPCR reagents at -20 ^{o}C.

Materials and equipment required (not provided with kit)

- BioChek Lysis Buffer A, catalogue numbers BP900 / BP910
- Mueller Kauffmann Tetrathionate Novobiocin Broth (MKTTn)
- Buffered Peptone Water (BPW)
- qPCR thermocycler (detection channels for FAM and HEX)
- Vortex mixer (x2)
- Mini-centrifuge (x2)
- Heating block capable of reaching 100 °C
- PCR plate-spinner (recommended)
- Disposable filter-tips for volumes of 1 – 1000 µl
- Single, 8 or 12 channel pipette
- DNase/RNase free 2.0 ml tubes for preparation of reaction mix
- Plates for PCR reaction (suitable for use with your qPCR thermocycler)
- Heat resistant sealers for plate
- Disposable powder free gloves
- Refrigerator and freezer

Warnings and precautions

1. Wear powder free disposable gloves at any stage of running the assay and/or sample preparation, change those gloves if you think they might be contaminated.
2. Handle all reagents with care.
3. Treat all biological materials as potentially biohazardous, including all field samples.
4. Never pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
5. This kit is for ***in vitro*** use only.
6. Strict adherence to the test protocol will lead to achieving best results.
7. Dedicate one airspace for kit storage/reagent preparation (Room 1, clean room) and another airspace (Room 2) for running the assay and sample preparation. A third airspace is optional (Room 3) for dedicated PCR amplification/running the assay. Alternatively, the use of a laminar flow cabinet or UV hood is recommended.
8. Never move any materials and equipment between the different PCR suites.

9. Decontaminate PCR laboratories/workplaces periodically with bleach ≥ 5 % (or alternative nucleic acid decontaminant) and UV light (optional) after testing. When using bleach make a fresh solution every day.
10. Assays should be performed by qualified laboratory personnel only.
11. Always use a positive control and a negative control for every PCR run.
12. Adhere to the expiration date of the qPCR kit and lysis buffers.
13. It is recommended to follow the requirements as described in the standard ISO 22174 "Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food pathogens – General requirements and definitions".
14. For quality results it is recommended to comply with Good Laboratory Practice (refer to ISO 7218 standard).
15. It is recommended to follow ISO 6887 standard for the sample preparation guidance.

Recommended work flow 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 - 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.

Sample preparation and DNA extraction protocol

For food products, feed products and production environmental samples:

- Enrichment in BPW (1/10) for 16 – 20 hours at 37 °C ± 1 °C.

For primary production samples:

- Enrichment in BPW (1/10) for 16 – 20 hours at 34 °C – 38 °C.
- Subculture in MKTTn broth (1 ml BPW + 10 ml MKTTn) for 24 h ± 3 h at 41.5 °C ± 1 °C.
- To reduce the risk of PCR inhibitions it is recommended to let the broth decant for approx. 10 min after vortexing MKTTn and before sampling DNA extraction.

DNA extraction on 100 µl enriched BPW for food, feed and production environmental samples.
DNA extraction on 100 µl subcultured MKTTn for primary production samples.
For DNA extraction protocol follow product insert for Lysis Buffer A (catalogue numbers BP900 / BP910).
In case of inhibition, a 1/10 dilution of the lysate in PCR grade water must be performed.
It is recommended to retain enriched broths for potential confirmatory testing. Enriched broth should be stored at 2 – 8 °C.
Extracts can be stored at 4 °C or frozen at -20 °C for several months or longer provided Stabilization Buffer has been added (Lysis Buffer A, catalogue numbers BP900 / BP910).

Test protocol for Salmonella qPCR

Reagent preparation

Room 1 – Clean Room

1. Defrost reagents at room temperature.
2. Vortex reagents thoroughly and briefly spin to remove any residues from the lid.
3. Calculate total volumes of DNA Mastermix I and PP/IC required for all reactions (reaction mix). Do not forget to include reactions for controls (minimum one positive and one negative).

For one 25 µl PCR reaction, the mixture is as follows:

DNA Mastermix I (black cap)	12.5 µl
PP/IC (yellow cap)	7.5 µl
Sample (DNA extract)	5.0 µl

4. Place the total volume of required DNA Mastermix I into a clean microtube.
5. Add the total volume of required PP/IC to the microtube.
6. Vortex microtube to mix thoroughly, and briefly spin to remove any residues from the lid.
7. Dispense 20 µl of reaction mix into each sample and control well of a microplate.
8. Optional Step: Add 5.0 µl of Negative control (blue cap). This is a reagent and environmental control.
9. Cover plate and take into PCR room 2.

Room 2/3 – Extraction / amplification room

1. Add 5.0 µl of Positive control (red cap) into control well(s).
2. Add 5.0 µl of Negative control (blue cap) into control well(s).
3. Add 5.0 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 program in the table (qPCR program at normal ramp speed).

Note: Do not use fast mode

Temperature	Time	No. of cycles
95 °C	3 mins	1
95 °C	15 sec	40
60 °C	60 sec	
Data collection (@ 60 °C): FAM = Salmonella HEX = Internal Control		

When the run is finished, remove the plate from the qPCR instrument and discard it without removing the seal.

Alternative channel names for the reporter dyes:
FAM: no alternative name
HEX: VIC, Yakima Yellow, CAL Fluor Orange 560, Alexa 532

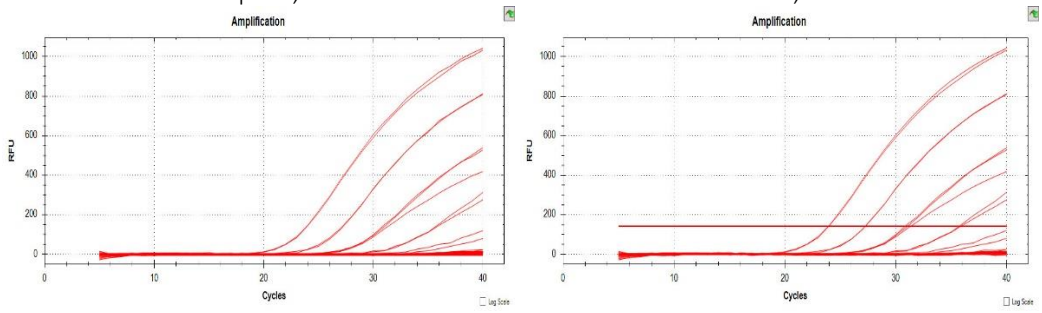
Validation and interpretation

Suggested analysis settings for validated thermocyclers

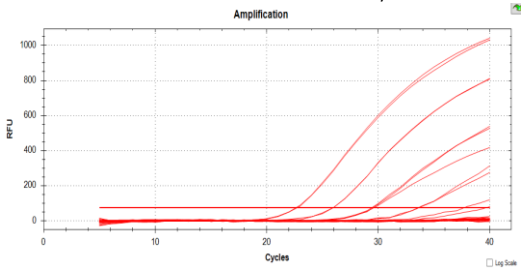
Bio-Rad CFX96™	Applied Biosystems® 7500	Stratagene Mx3005P™	Roche LightCycler® 480
Fluorescense drift correction: yes	Use passive reference: none	Amplification-based threshold	Analysis method: Abs quant/2nd derivative max
Cycles to analyse: 5 – 40	Baseline cycles: 3 – 15	Adaptive baseline	Do not use color compensation

Setting thresholds in the cycler software

Go to the part of the software 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 nr 20.
Look at which cycle the first formed curve goes up in 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 the HEX channel.
Important note: after setting the thresholds for both channels, keep using those thresholds for all future PCR runs.

Validation of assay run

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

	Salmonella (FAM) Cq values	IC (HEX) Cq values	Interpretation
Positive Control	22-33	Not considered	Valid Control
Negative Control	N/A*	<38	Valid Control

*No Cq value

Repeat the PCR plate in the event of control failure.

Validation and interpretation of sample results

Salmonella (FAM) Cq values	IC (HEX) Cq values	Interpretation
<40	Not considered	positive sample Salmonella
N/A*	<38	negative sample
N/A*	N/A*, >38	invalid well

*No Cq value

For final diagnosis qPCR positive results should be considered presumptive and confirmed by standard reference methods or alternative tests for Salmonella.

In the context of NF VALIDATION all samples identified as positive by the method Salmonella Species DNA Test Kit must be confirmed by using the protocol of the ISO 6579-1 method.

BCK 40/01-07/19

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

http://nf-validation.afnor.org/en

The Salmonella Species DNA Test Kit is certified NF VALIDATION as an alternative method to reference method NF EN ISO 6579-1, for the detection of Salmonella species in food products (RTE, RTRH, meat products, ingredients and specific foods), feed products, production environmental and primary production samples. The validation followed the ISO 16140-2 (2016) protocol, and includes the use of Bio-Rad CFX96™, Applied Biosystems® 7500, Roche LightCycler® 480 and Stratagene Mx3005P™systems. For more information about software used during validation and end of validity of the NF CERTIFICATION, please refer to the certificate BCK 40/01-07/19 available on the website mentioned above. Test portions weighing more than 25 g have not been tested in the NF VALIDATION study.

KI/MP104REV05

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Symbol Glossary

REF	Catalogue number		Expiry date
EC REP	Authorised representative in the European Community	LOT	Batch number
	Manufacturer	SN	Serial number
	Date of manufacture		Lower limit of temperature
	Consult instructions for use	CONTROL +	Positive control
CONTROL -	Negative control		