Salmonella qPCR Controls

BioChek qPCR reagents

Catalogue Number MP404

Product description

Salmonella qPCR controls are designed to be used with BioChek qPCR reagents. They can be used in combination with the following BioChek products for a complete Salmonella qPCR solution:

- DNA Mastermix (BP300)
- Salmonella qPCR primer probe – internal control (MP304)
- Lysis Buffer A (BP900 / BP910)

Reagents and materials provided

1. qPCR Negative Control, 2 vials (Blue Cap), molecular grade water (60 µl). This is sufficient for 20 BioChek qPCR reactions.
2. 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) This is sufficient for 20 BioChek qPCR reactions.

Storage conditions: Upon receipt, store at -20 ^{o}C (+/- 5 ^{o}C)

Materials required but not provided for running PCR

- qPCR thermocycler (detection channels for FAM, HEX, Texas Red and CY-5)
- Vortex mixer (x2)
- Mini-centrifuge (x2)
- PCR plate-spinner
- 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
- DNA extraction method
- Plates for PCR reaction (suitable for use with your qPCR thermocycler)
- Heat resistant sealers for plate
- Disposable powder free gloves
- DNA primer/probe
- DNA Mastermix

Reporter dyes (if used with BioChek qPCR reagents)

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

Example protocol

The following can be used in testing with BioChek products BP300 and MP304

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 Mastermix 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:

Mastermix 2X (black cap - BP300)	12.5 µl
PP/IC (yellow cap – MP304)	7.5 µl
Sample (DNA extract)	5.0 µl

4. Place the total volume of required Mastermix 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 – MP404). This is a reagent and environmental control.
9. Cover plate and take into PCR room 2.

Room 2 – Extraction / amplification room

1. Add 5.0 µl of Positive control (red cap – MP404) into control well(s).
2. Add 5.0 µl of Negative control (blue cap – MP404) 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: <u>Do not</u> 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.

Suggested analysis and threshold settings

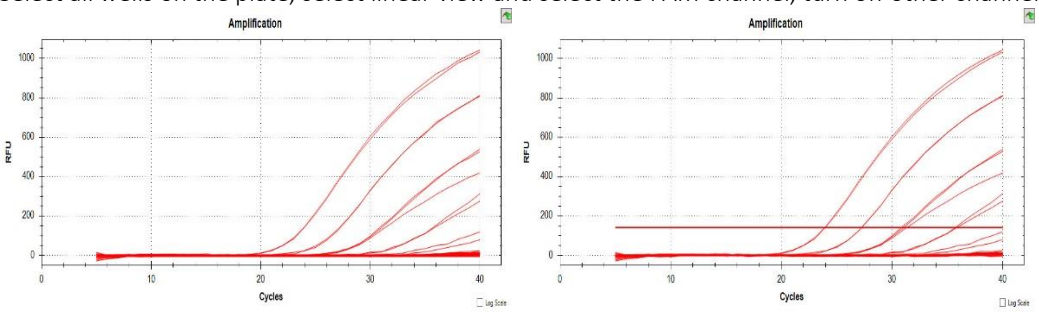
Analysis settings for BioRad CFX96, ABI-7500 and Agilent Mx3005p cyclers.

BioRad CFX96:	Lifetech ABI-7500:	Agilent Mx3005P:
Fluorescense drift correction: yes	Use passive reference: none	Amplification-based threshold
Cycles to analyse: 5 – 40	Baseline cycles: 3 – 15	Adaptive baseline
Software version 3.1	Software version 2.06	Software version 4.10

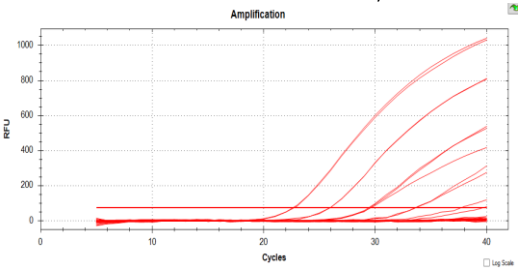
Other PCR machines can be used, contact BioChek for further information

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 number 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 controls (when using Salmonella qPCR PP/IC MP304 and Mastermix BP300)

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.

Interpretation of sample results (when using Salmonella qPCR PP/IC MP304 and Mastermix BP300)

Salmonella (FAM) Cq values	IC (HEX) Cq values	Interpretation
<40	Not considered	Positive sample for Salmonella
N/A*	<38	Negative sample
N/A*	N/A*, >38	Invalid well**

*No Cq value

**When a sample is negative for Salmonella and the internal control is out of range, the assay is invalid for this particular sample, and should be repeated with a 1/10 dilution of the extract or a new DNA extract.

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.

Positive samples must be confirmed by culture. The enriched sample used for the rapid assay should be transferred into MSRV and follow the isolation and identification procedures in PS Standard B (3)(b)(1)(ii-vi). The culture process must be started within 24 hours of the positive screening test.

For technical assistance contact your BIOCHEK area manager or distributor or visit our website.

RI/MP404REV02

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Symbol Glossary

REF	Catalogue number		Expiry date
EC REP	Authorised representative in the European Community	LOT	Batch number
	Manufacturer	Σ	Content
	Date of manufacture	IVD	In vitro diagnostic
	Consult instructions for use		Lower limit of temperature