PhoenixDx Kit

Salmonella enterica

For Research Use Only.

Procomcure Biotech GmbH
Breitwies 1
5303 Thalgau
T.: +43 6246 73075
F.: +43 6246 73058
office@procomcure.com
www.procomcure.com

Content

1. Product Description

PhoenixDx Kit *Salmonella enterica* is a detection system for *Salmonella enterica* DNA based on real-time PCR. The kit allows for rapid and sensitive detection of *Salmonella enterica* DNA previously purified from various samples.

PhoenixDx Kit *Salmonella enterica* detects invA (invasion protein A), a gene highly specific for members of the genus *Salmonella* and essential for their full virulence. Presence of invA in the purified DNA sample is measured through a probe-specific FAM-signal at 530 nm.
The kit also contains a PCR positive control (PPC, measured through a Cy5-Signal at 667 nm). The PPC helps to exclude false-negative test results e.g. through flawed DNA extraction or incorrect reaction setup. For well-to-well normalization, ROX is included as a passive reference dye. This helps to compensate for pipetting inaccuracies and improves overall data quality. However, the kit can be used without ROX normalization as well. Before starting the experiment, please make sure that your device is equipped and calibrated for FAM, Cy5 (and ROX if required).

2. Pathogen Information

Salmonella are a member of the gram-negative *Enterobacteriaceae* family, the genus only consisting of two species: *S. enterica* and *S. bongori*. While *S. bongori* preferably infects cold blooded animals, *S.enterica* are responsible for most infections in warm blooded animals. Infections in warm-blooded animals are generally caused by *S.enterica* subspecies *enterica;* non-typhoidal serotypes are the more common ones and cause a gastrointestinal disease.
Furthermore, *Salmonella* can infect a broad range of animals and are transferable between humans and animals. The most common serotypes among animals are*: S. Typhimurium, S. Dublin, S. Abortusovis*, and *S. Enteriditis*.

3. Real-Time PCR for Pathogen Detection

In real-time PCR, a DNA sequence highly specific for the designated pathogen is amplified. The emerging PCR product is detected via an increase in fluorescence signal. Pathogen detection via real-time PCR provides highly specific and sensitive detection of pathogen DNA and can help to reduce bacterial waste and pathogen exposure.

4. General Considerations

Before starting, please review our guidelines for a successful experiment:

- Always include a control reaction with water replacing the DNA sample. This will help to detect possible DNA contamination.
- *Optional*: include a negative control in the DNA isolation step e.g. with medium/water instead of sample material.
- Be careful when handling positive control and sample material
- Make sure that positive controls and materials are stored separately from the other reagents and setup the final reaction in a separate workspace.
- Always pipet positive controls last.
- Decontaminate workspace and equipment on a regular basis.
- Use sterile filtered tips for all steps.

5. Kit Components

Label	Content	Amount		Storage at
		50 rxn	100 rxn	
S. enterica Assay Mix	Primer & FAM-Probe for *S. enterica* detection	1x 50 µl	2x 50 µl	-20°C
PPC	Primer, Cy5-Probe & template for PPC detection	1x 50 µl	2x 50 µl	-20°C
S. enterica Positive Control	Control DNA (~ 10 000 copies / µl)	1x 25 µl	1x 25 µl	-20°C
PCR Reaction Mix	Reaction Mix	1x 500 µl	2x 500 µl	-20°C
Water	Water	1x 1000 µl	1x 1000 µl	-20°C to 4°C

All components are stable to the expiry date indicated on the tubes. Avoid repeated freezing and thawing and protect all components from light.

6. Additional Material Required

- Suitable Reagents / Devices for DNA isolation
- Disposable powder-free gloves
- Sterile filtered pipette tips
- Benchtop Centrifuge for PCR Tubes / Plates
- Suitable PCR Plates or Tubes and corresponding optical closing materials
- Real-Time PCR Cycler able to detect FAM, Cy5 (and ROX, if required)

7. Setting Up the Experiment

Always include a negative control, a positive control and an extraction negative control in your PCR run. We highly recommend performing all reactions at least in duplicates as it facilitates interpretation of your results and increases reliability.

7.1 Pipetting Scheme

		per 20 µl-reaction
Mastermix (mix thoroughly)	Water	3.0 µl
	PCR Reaction Mix	10.0 µl
	S. enterica Assay Mix	1.0 µl
	PPC	1.0 µl
	Total Volume	**15.0 µl**
PCR Assay	Mastermix	15.0 µl
	Sample[1]	5 µl
	Total Volume	**20.0 µl**

[1] 1-8 µl of sample can be used. Make sure to adjust the amount of water accordingly.

Positive control: for positive control, prepare 1 µl of the supplied S. enterica Positive Control + 4 µl water and use instead of sample material. If required, the *S. enterica* Positive Control can be diluted 1:10. Make sure to prepare a sufficient amount of mastermix for all planned reactions.

7.2 Setting Up the Cycling Program

For precise programming instructions please refer to the manufacturer's guidelines of your device. It is recommended to start the device before setting up the PCR reactions to allow it to reach operating temperature.

Settings: **FAM** for detection of *Salmonella enterica* DNA
Cy5 for detection of the PPC
ROX for passive reference
Sample Volume: **20 µl**

Cycling Program:

	Cycles	Temperature	Time
Step 1	1	94°C	5 min
Step 2	55	94°C	15 sec
Step 3		55°C	70 sec

8. Analysis

For analysis of your results, select the FAM channel for *S. enterica*, the Cy5 channel for PPC. Samples with a positive Ct value can be considered positive. Possible outcomes are:

FAM- & Cy5-Signal: DNA of *Salmonella enterica* was present in the reaction. The sample is considered positive.

FAM- but no Cy5-Signal: The sample is considered positive. A high amount of *Salmonella enterica* DNA in the sample may inhibit the amplification of the PPC.

No FAM- but Cy5-Signal: The sample does not contain a detectable amount of *Salmonella enterica* DNA. The sample is considered negative.

No FAM- & no Cy5-Signal: PCR was probably inhibited, or the DNA extraction was not successful. Results cannot be interpreted.

	Ct/Cp (FAM) S. enterica target	Ct/Cp PPC	Interpretation
Negative Control	/	32 ± 2	Valid
S. enterica Positive Control	27.0-29.0	32 ± 2	Valid
S. enterica Positive Control 1:10	30.0-33.0	32 ± 2	Valid
(optional) DNA Isolation Neg. Control	/	32 ± 2	Valid
Negative Sample	/	32 ± 2	Valid
Positive Sample	+	32 ± 2	Valid

9. Troubleshooting

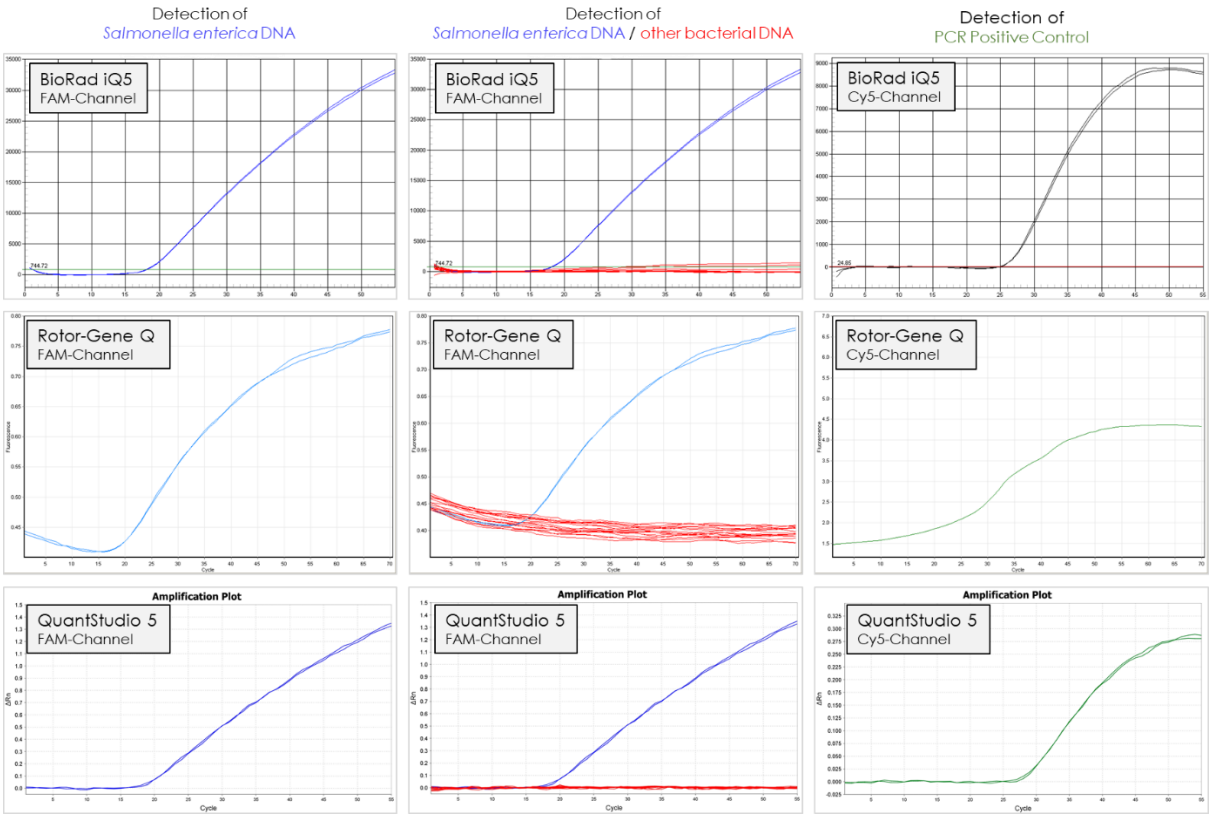
No FAM-Signal in the positive control

Check the cycling protocol; an incorrect program can prevent amplification.
Check your reaction setup and repeat the PCR.

No FAM- and Cy5-Signal	PCR was inhibited. Check your DNA isolation process for issues. Repeat the PCR with a diluted DNA sample to dilute potential inhibitor carryovers from purification. Check the cycling protocol; an incorrect program can prevent amplification.
FAM-Signal in the negative control	A DNA contamination is present. Repeat the reaction with fresh reagents. If necessary, you can make aliquots of the PCR Reaction Mix and the water before first use of the kit. Pipet positive controls last. Decontaminate your equipment on a regular basis.
FAM-Signal in the negative extraction control.	A DNA contamination occurred during DNA isolation. Repeat isolation with fresh reagents and make sure to clean and decontaminate your equipment on a regular basis.

10. Exemplary Results

The following graphics show exemplary outcomes of experiments using PhoenixDx Kit for the detection of *S. enterica*. As negative controls, bacterial DNA from other pathogens (such as *Cronobacter sp., Legionella sp*. and *Listeria sp*.), water or human genomic DNA was used instead of *Salmonella enterica* DNA.



11. Specifications

11.1 Sensitivity

The analytical sensitivity of PhoenixDx Kit *Salmonella enterica is* ***4*** *target copies per PCR reaction.*
The limit of detection (LoD95, smallest number of target DNA copies that can be detected in 95% of tests) is ***20*** *target copies per reaction. The LoD95 was determined by several replicates in the range of detection limit.*

11.2 Specificity

Specificity is ensured by the design of highly selective primers and probes for *Salmonella enterica* DNA. Primers and Probes were evaluated for possible homologies by sequence comparison analysis. For several common food pathogens (such as *Cronobacter sp., Legionella sp*. and *Listeria sp*.), primers and probes were tested in real-time PCR experiments for target specificity.