



For Laboratory Use Only

REF	PCCSKU15001		50
REF	PCCSKU15002		100



Procomcure Biotech GmbH
Breitwies 1
5303 Thalgau - Austria
T.: +43 6229 39608
office@procomcure.com
www.procomcure.com



Content

1. Product Description	3
2. Pathogen Information	3
3. Real-Time PCR for Pathogen Detection	3
4. General Considerations	3
5. Kit Components	4
6. Additional Material Required	4
7. Setting Up the Experiment	4
7.1 Pipetting Scheme	4
7.2 Setting Up the Cycling Program	5
8. Analysis	5
9. Troubleshooting	5
10. Exemplary Results	6
11. Specifications	7
11.1 Sensitivity	7
11.2 Specificity	7
12. Related Products	7

NOTICE TO PURCHASER: LIMITED LICENSE

The purchase of this product includes a limited, non-transferable immunity from suit under patent claims for using only this amount of product solely in Food Testing Applications and Industrial Microbiology Applications, including reporting results of purchaser's activities for a fee or other commercial consideration, and also for the purchaser's own internal research. No right under any other patent claim is conveyed expressly, by implication, or by estoppel. This product is for laboratory use only.

LICENSE AGREEMENT FOR THE PHOENIXDX MANUAL:

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. Procomcure Biotech grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this manual.
2. Other than expressly stated licenses, Procomcure Biotech makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. Procomcure Biotech specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agrees not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. Procomcure Biotech may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

1. Product Description

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

PhoenixDx *Salmonella spp.* 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* is 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. Enteritidis*.

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	
Salmonella spp. Assay Mix	Primer & FAM-Probe for <i>Salmonella</i> spp. 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
Salmonella spp. 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

Mastermix (mix thoroughly)		per 20 µl-reaction
	Water	3.0 µl
	PCR Reaction Mix	10.0 µl
	<i>Salmonella</i> spp. 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 *Salmonella* spp. Positive Control + 4 µl water and use instead of sample material. If required, the *S. spp.* 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* spp. 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 *Salmonella* spp., the **Cy5** channel for PPC. Samples with a positive Ct value can be considered positive. Possible outcomes are:

FAM- & Cy5-Signal:

DNA of *Salmonella* spp. 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* spp. 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* spp. 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) <i>Salmonella</i> spp. target	Ct/Cp PPC	Interpretation
Negative Control	/	32 ± 2	Valid
<i>Salmonella</i> spp. Positive Control	27.0-29.0	32 ± 2	Valid
<i>Salmonella</i> spp. 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.

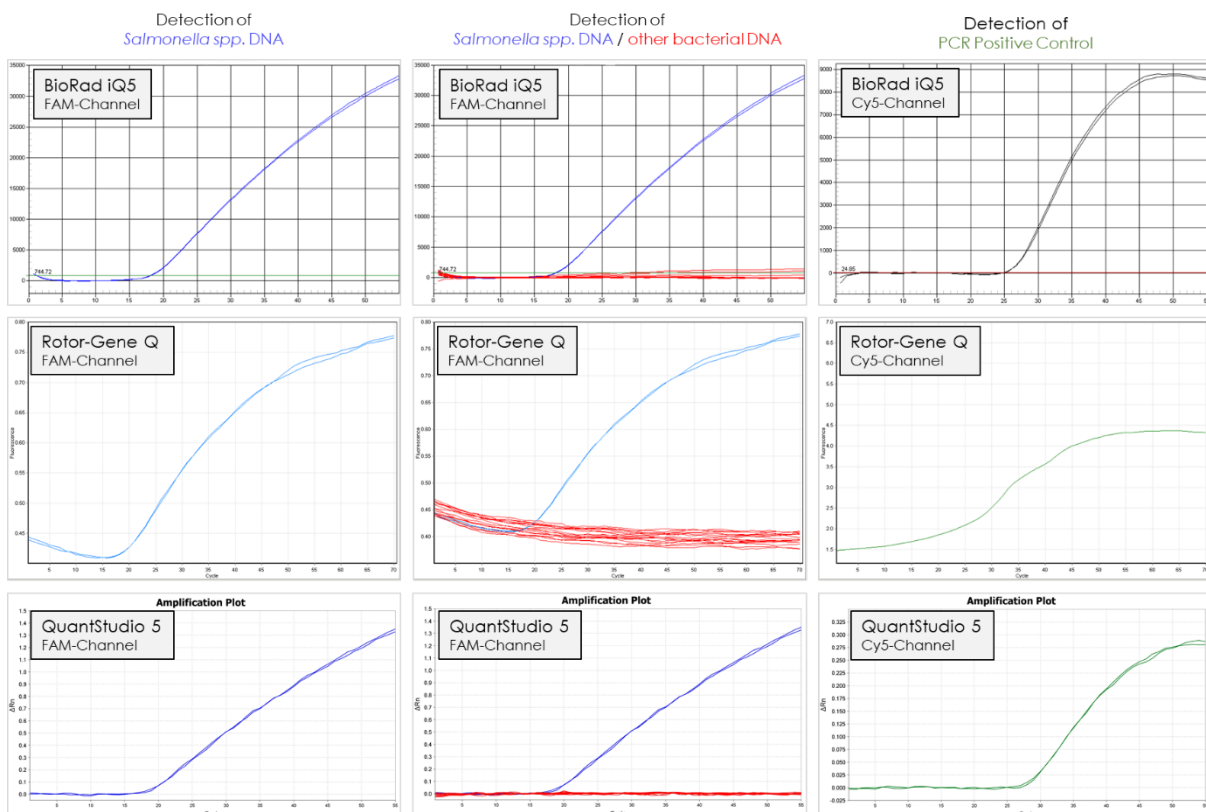
Decontaminate your equipment on a regular basis.

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 for the detection of *Salmonella* spp.. 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* spp. DNA.



11. Specifications

11.1 Sensitivity

The analytical sensitivity of PhoenixDx *Salmonella* spp. 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* spp. 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.

12. Related Products

Product	Size	SKU
2X Magic SYBR Mix	2 ml	PCCSKU1107
	5 ml	PCCSKU1108
2X Magic Probe Mix	2 ml	PCCSKU1109
	5 ml	PCCSKU1110
1Step Magic SYBR Kit	200 rxn/20 µl	PCCSKU1201
	600 rxn/20 µl	PCCSKU1202
1Step Magic Probe Kit	200 rxn/20 µl	PCCSKU1203
	600 rxn/20 µl	PCCSKU1204

