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SCAN BEC
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QPCRScan
Salmonella

Test kit for qualitative detection of *Salmonella* spp. by Real-Time PCR

Product-No. 91289

Kit for 96 Real-Time PCR reactions

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Introduction

QPCRScan - *Salmonella* Real-Time Kit is a rapid molecular test system for the qualitative detection of bacteria of the genus *Salmonella*, including the detection of all serovars like *S. enteritidis, S. typhimurium, S. typhi* and *S. paratyphi*.

Working Principle

This kit is based on a real-time PCR method with specific primers and fluorescence-labeled probes. A flexible use in all common PCR instruments is possible. Probes specific for *Salmonella* are labeled with the fluorophore FAM and it for the internal control is labeled with fluorophore HEX. Please ensure that your instrument which you are using can measure these two dyes. The kit is suitable for the detection of *Salmonella* after pre-enrichment according to EN ISO 6579: 2002+ Amd 1:2007 and for the confirmation of *Salmonella* spp. colonies.

Safety

All reagents contained in the test kit are for *in vitro* use only. Handling of the kit components and disposal of waste should be performed according to standard laboratory safety guidelines.

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Kit components

The QPCRScan - *Salmonella* real-time PCR contains reagents for 96 reactions. The kit components with their color code and storage temperature are listed in the following table:

Table 1: Kit components with color code and storage temperatures

Component	Color code	Storage	Volume/ Reactions	Store after dissolving at
Re-Buffer		4°C	1.1 ml	
PCR-grade H_2O		4°C	2 ml	
qPCR-LyoMix SA		4°C	96 rxn.	-20°C
LyoProbe SA		4°C	96 rxn.	-20°C
IAC		4°C	96 rxn.	-20°C
SA Positive Control		4°C	100 µl	-20°C

Storage

The QPCRScan *Salmonella* contains lyophilized PCR solutions and should be stored until dissolving at 4°C. Once the reagents were dissolved, they should be stored at -20°C.

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Additional Equipment and Materials

(Required, not supplied with kit)

- DNA-Extraction kit or validated in-house method
- Microcentrifuge
- Micropipettes variable for 10 µl, 100 µl and 1000 µl and sterile filter tips to avoid cross contaminations
- Reaction tubes (0.2 ml and 1.5 ml)
- Realtime PCR Cycler with FAM channel (518 nm) and HEX channel (536 nm)

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DNA-Extraction

- 50 µl to 2 ml pre-enrichment culture for DNA extraction can be used after at least 20h enrichment according to EN ISO 6579: 2002+ Amd 1:2007.
- The DNA can be isolated by using DNA extraction kit to isolate bacterial DNA or by a validated in-house method.
- Make sure that the sample contains a minimum quality of DNA (10ng).
- DNA extraction from suspect single colonies can be performed by suspending a single colony in 0.5ml sterile water and incubation of the suspension at 95°C.
- 1 µl supernatant after 15 min centrifugation at 13000 x g is used in PCR.

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Real-time PCR

Precautions

- Sample and PCR reaction mixtures should prepared in separate areas
- While pipetting the reaction mixtures hold tubes on ice or in a cold block.
- Keep reagents cool and dark and mix gently before each use.
- Use single-use pipette tips to avoid cross-contamination.
- Wear disposable powder free gloves.
- Close tubes immediately after use and store them at the temperatures specified in the table above.
- Samples and controls (positive and negative) should be tested together for accurate results.
- Do not mix or replace components from test kits of different charges.
- Do not use the test kit after the expiration date listed on the package.

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Preparations

1. Dissolve lyophilized reagents with the appropriate solution and volume specified in the following table:

Table 2: Dissolving volumes

Component		Volume	Reagent
qPCR-LyoMix SA		1000 µl	Re-Buffer
LyoProbe SA		110 µl	PCR-grade H_2O
IAC		1250 µl	PCR-grade H_2O

2. Mix resuspended PCR reagents thoroughly and briefly centrifuge vials before opening.

! **Note:**
After dissolving lyophilized reagents, please store at -20°C.

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Real-time Master Mix

1. Prepare Master Mix for the appropriate number of samples (including positive and negative controls) as shown in Table 3.

Table 3: PCR mixture for one sample

Components		Volume
qPCR-LyoMix SA		10 µl
LyoProbe SA		1.0 µl
IAC		1.0 µl
PCR-grade H_2O		5.5 µl
Total PCR mix		**17.5 µl**

2. Add 17.5 µl Master Mix to all reaction tubes.
3. Add 2.5 µl of Sample-DNA (10-100ng) and positive/negative controls into the appropriate labelled reaction tubes.

DNA (of sample, positive & negative controls)	2.5 µl
Final volume	**20.0 µl**

! **Note:**
For each assay set use:
- Extraction control (not supplied with kit)
- Positive control (SA Positive Control DNA)
- No template control (PCR-grade H_2O instead of sample DNA)

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PCR Cycler Settings

PCR reactions are performed according to the following temperature profile:

Table 4: Program for standard cycling

Step	Temperature	Time	Cycles	Channel	Acquisition
1	95°C	2 min	1	FAM & HEX	no
2	95°C	30 sec	45		
	52°C	45 sec			
	40°C	45 sec			**yes**

If your lab has a Fast-enabled instrument, you can run the Fast-Cycling protocol:

Table 5: Program for Rapid cycling

Step	Temperature	Time	Cycles	Channel	Acquisition
1	95°C	2 min	1	FAM & HEX	no
2	95°C	4 sec	45		
	52°C	20 sec			
	40°C	20 sec			**yes**

! **Note:**
For some real-time PCR instruments the usage of a passive reference dye or the type of the quencher has to be specified. Please Select BHQ as Quencher and 'no reference' dye

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Data Interpretation

Real-time PCR data are figured as a sigmoidal-shaped amplification plots (when using a linear scale), in which fluorescence is plotted against the number of cycles.

Salmonella specific DNA is detected at the 518nm (FAM). The inhibition control is detected at 553nm (HEX). Samples with a detectable Ct-value are positive (Table 6).

Table 6: Results and data interpretation

***Salmonella* (FAM) (Samples and Positive Controls)**	**IAC (HEX)**	**Sample Result**
Positive	Positive	**Positive**
Positive	Negative	**Positive**
Negative	Positive	**Negative**
Negative	Negative	**Inhibition**

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Troubleshooting Guide

Table 7: Troubleshooting

Result	Possible Reason	Suggestions
No amplification for Internal Amplification Control (IAC)	Inhibition of PCR-Reaction	Dilute samples and/or purify sample DNA using a purification Kit
Positive 'No template control' (NTC)	Cross-Contamination with positive DNA	Check your precautions and repeat your experiments / Replace all critical solutions
No amplification is observed, neither from Sample DNA nor from positive controls	No data acquisition has been selected	Check the cycling programs
	Wrong Cycling program	Adjust/select the correct program
	Incorrect detection channel has been selected	Set Channel settings to FAM and HEX/VIC
	Pipetting errors or missing addition of reagents	Repeat PCR run and pay attention for the correct pipetting of the Master Mix
Positive extraction control	Carry-over during DNA extraction and/or contamination of used reagents	Repeat PCR extraction and check all solutions

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Contact Information

We provide a qualified technical service. If you have any questions or problems with the handlings of our PCR kit, do not hesitate to contact us.

Internet
sigma-aldrich.com

Technical Service:
techservice@sial.com

QPCRScan (R&D):
contact@scanbec.de

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Related Products

Kat. Nr.	Name
QPCRScan Real-Time PCR Detection Kits	
05829	**QPCRScan***Campylobacter*
06040	**QPCRScan***Listeria monocytogenes*
06238	**QPCRScan***Listeria*
91289	**QPCRScan***Salmonella*
HybriScan®***I***	
79742	HybriScan®***I*** *Brettanomyces*
19503	HybriScan®***I*** Candida albicans
76545	HybriScan®***I*** *E. coli*
75724	HybriScan®***I*** *Lactobacillus brevis*
80065	HybriScan®***I*** *Lactobacillus buchneri*
86827	HybriScan®***I*** *Lactobacillus lindneri*
49417	HybriScan®***I*** *Legionella pneumophila*
77007	HybriScan®***I*** *Leuconostoc*
49712	HybriScan®***I*** *Listeria monocytogenes*
42876	HybriScan®***I*** *Megasphaera*
33018	HybriScan®***I*** *Pectinatus cerevisiiphilus*
73582	HybriScan®***I*** *Pectinatus frisingensis*
67289	HybriScan®***I*** *Pectinatus damnosus*
HybriScan®***D***	
62533	HybriScan®***D*** Beer
56917	HybriScan®***D*** Campylobacter
68301	HybriScan®***D*** Drinks
96343	HybriScan®***D*** *E. coli*
12838	HybriScan®***D*** *Enterobacter sakazakii*
59744	HybriScan®***D*** Lactobac
16593	HybriScan®***D*** *Legionella*
07190	HybriScan®***D*** *Legionella pneumophila*
55661	HybriScan®***D*** *Listeria*
49699	HybriScan®***D*** *Listeria monocytogenes*
55662	HybriScan®***D*** *Salmonella*
02349	HybriScan®***D*** Total Bacterial Count
78436	HybriScan®***D*** Waste Water Total Bacterial Count (Modul 1)
04447	HybriScan®***D*** Waste Water *Microthrix parvicella* (Modul 2)
61397	HybriScan®***D*** Yeast

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Version 2, March 2016