LightMix® Kit *Salmonella* spp.
Cat.-No. 40-0308-16

Kit with reagents for the detection of *Salmonella* spp. DNA using the Roche Diagnostics LightCycler® 1.x / 2.0 / 480 II Instruments.

Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do NOT freeze!**

Instructions for use with the LightCycler® 1.x / 2.0 Instruments see pages 4-5
Instructions for use with the LightCycler® 480 II Instrument see pages 6-7

1. Introduction

*Salmonella* is a genus of rod shaped Gram-negative bacteria that causes foodborne illness, typhoid fever and paratyphoid fever. The *Salmonella* genus is composed of two species, six subspecies and 2,500 serovars. In particular, *Salmonella enterica* serotypes *Typhi* and *Paratyphi* are important causes of enteric fever in developing countries that lack adequate sewage disposal and water treatment facilities. Other salmonellae are frequent causes of foodborne illness contracted from poultry, raw eggs, improperly stored food or the handling of reptiles and amphibians, which host *Salmonella*. Human *Salmonella* infections usually resolve in 5-7 days and often do not require treatment unless the patient becomes severely dehydrated or the infection spreads from the intestines.

The LightMix® Kit *Salmonella* spp. provides a fast, easy and accurate system to identify this target in a nucleic acid extract. A control amplification reaction acts as internal control (IC).

This LightMix® Kit is tested on the LightCycler® 2.0 / 480 II (96 well format) Instruments with Roche Diagnostics ‘LightCycler® FastStart DNA Master HybProbe’.

2. Description

A 194 bp fragment of the *prgK* gene\(^1\) of the *Salmonella* spp. genome is amplified with specific primers. The resulting PCR fragment is analyzed with hybridization probes labeled with LightCycler® Red 640 (detected in channel 640).


The PCR reaction is monitored by an internal control of 200 bp. This control does not interfere with the *Salmonella* spp. specific reactions. The amplification will usually fail in the presence of higher concentrated *Salmonella* spp. DNA samples (1,000 copies or higher) while displaying an amplification signal in negative and low-concentrated samples. Detection is recorded in channel 705.

The IC is supplied separately to allow running the assay in the presence or absence of the IC.

The use of a color compensation file generated with the Roche Diagnostics ‘LightCycler®-Color Compensation Set’ (LightCycler® 1.x Instrument) or with the Roche Diagnostics ‘LightCycler® Multicolor Demo Set’ is a prerequisite to run the duplex reaction.

The supplied standard row allows to determine the linear range of the reaction and to estimate the quantity of the target sequence in unknown samples.

For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler® 1.x Instruments to software version 4.1.
3. Set contents

6 Vials with green caps containing premixed lyophilized primers and probes for 16 PCR reactions each of *Salmonella* spp.
6 Vials with white caps containing the internal control (IC)
1 Standard row with 6 lyophilized cloned plasmid standards of *Salmonella* spp. from 10<sup>1</sup> to 10<sup>6</sup> target equivalents per reaction
1 Sealing foil for the standard row

4. Additional reagents and items required

*Roche Diagnostics:*
- LightCycler® FastStart DNA Master HybProbe
  - Cat.-No. 03 003 248 001
- LightCycler® Multicolor Demo Set
  - or LightCycler® Color Compensation Set (LightCycler® 1.x Instrument)
  - Cat.-No. 03 624 854 001
  - Cat.-No. 12 158 850 001
- High Pure PCR Template Preparation Kit
  - Cat.-No. 11 796 828 001
- LightCycler® Capillaries (20 µl) (LightCycler® 1.x / 2.0 Instruments)
  - or LightCycler® 480 Multiwell Plate 96, white (LightCycler® 480 II Instrument)
  - Cat.-No. 04 929 292 001
  - Cat.-No. 04 729 692 001

5. Product characteristics

PCR results are obtained within 50 minutes (50 cycles and melting curve) with the LightCycler® 1.x / 2.0 Instruments and within 80 minutes (50 cycles and melting curve) with the LightCycler® 480 II Instrument.

*Sensitivity*
These reagents detect 10 copies of *Salmonella* spp. DNA using the Roche ‘LightCycler® FastStart DNA Master HybProbe’ with the LightCycler 1.x / 2.0 / 480 II Instruments (in an exemplary system, using cloned targets as reference).

*Measuring range*
The linear measuring range of the assay is 10<sup>2</sup> to 10<sup>6</sup> copies of *Salmonella* spp. DNA using the Roche ‘LightCycler® FastStart DNA Master HybProbe’ with the LightCycler 1.x / 2.0 / 480 II Instruments.

*Storage and Stability*
- Lyophilized reagents are stable for at least 3 months after shipment if stored protected from light at room temperature (18-25°C).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days if stored protected from light and refrigerated (4°C).

6. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 / 480 II Instruments.

Start programming before preparing the solutions. See the LightCycler® Instrument operator’s manual for details.

*Sample material:* Use aqueous nucleic acid preparations (e.g. Roche Diagnostics ‘High Pure PCR Template Preparation Kit’).

*Negative control:* Always run at least one no-template control (NTC) - replace the template DNA with water.

*Positive control:* Run a positive control - replace the template DNA with the provided control DNA. For quantification always use a fresh solution prepared from the provided standard row (single use only).
6.1. Preparation of parameter-specific reagents and reagents for the IC (16 reactions):

One reagent vial with a **green** cap contains all primers and probes to run 16 LightCycler® reactions for *Salmonella spp.*

One reagent vial with a **white** cap contains all primers, probes and DNA to run 16 LightCycler® reactions for the IC.

**Add 66 µl** PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

► **Use 4 µl** reagent for a 20 µl PCR reaction.

This solution is stable at least five days when stored refrigerated at 4°C. Avoid prolonged exposure to light.

6.2. Preparation of the standard row

The target DNA is provided in 6 different quantities to yield from $10^1$ to $10^6$ target molecules in 5 µl once resolved. Start with the lowest concentrated standard (first tube from the extended lip). Use the pipette tip to punch a hole through the sealing foil. **Add 40 µl** PCR-grade water to each vial of the row. Mix the target DNA by pipetting the solution up and down 10 times.

► **Use 5 µl** standard for a 20 µl PCR reaction.

This standard solution is not long-term stable and will lose sensitivity under prolonged storage. Use only fresh prepared solutions as quantification references. Older solutions can be used as a qualitative standard (positive control). After adding the target DNA to the LightCycler® reaction mix use the provided sealing foil to close the vials in order to avoid changes in the concentration due to evaporation.

Please note that opening these vials may cause contaminations of the work-space (aerosol).

6.3. Preparation of the LightCycler® reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

<table>
<thead>
<tr>
<th>For use with the Roche FastStart Master</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single reaction</strong></td>
<td><strong>Component</strong></td>
</tr>
<tr>
<td>2.6 µl</td>
<td>water, PCR-grade (colorless cap, provided with the Roche Master kit)</td>
</tr>
<tr>
<td>2.4 µl</td>
<td>Mg$^{2+}$ solution 25 mM (blue cap, provided with the Roche FastStart kit)</td>
</tr>
<tr>
<td>4.0 µl</td>
<td>reagent mix (parameter specific reagents containing primers and probes, see 6.1.)</td>
</tr>
<tr>
<td>4.0 µl</td>
<td>IC mix (IC reagents containing primers, probes and DNA, see 6.1.)</td>
</tr>
<tr>
<td>2.0 µl</td>
<td>Roche Master (red cap, for preparation see Roche manual)</td>
</tr>
<tr>
<td><strong>15.0 µl</strong></td>
<td><strong>Volume of reaction mix</strong></td>
</tr>
</tbody>
</table>

To include the internal control **add 4 µl** of the IC reagent per reaction to the reaction mix.

To run the assay without the internal control substitute the 4 µl of IC components with 4 µl PCR-grade water.

Mix gently, spin down and **transfer 15 µl** each of the reaction mix to a LightCycler® capillary.

**Add 5 µl** of sample or standard to each capillary for a final reaction volume of 20 µl.

Start run.
7. LightCycler® 1.x / 2.0 Instrument

7.1. Programming

The protocol consists of four program steps

• 1: Denaturation: sample denaturation and enzyme activation
• 2: Cycling: PCR-amplification of the target DNA
• 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
• 4: Cooling: cooling the instrument

<table>
<thead>
<tr>
<th>Program Step</th>
<th>Denaturation</th>
<th>Cycling</th>
<th>Melting</th>
<th>Cooling</th>
</tr>
</thead>
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<tr>
<td>Parameter</td>
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<td>Quantification mode</td>
<td>Melting Curves mode</td>
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<td>Cycles</td>
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<td>50</td>
<td>1</td>
<td>1</td>
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<td>95</td>
<td>62</td>
<td>72</td>
</tr>
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<td>00:00:05</td>
<td>00:00:05</td>
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<tr>
<td>Ramp Rate [°C/s]</td>
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<td>20</td>
<td>20</td>
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<tr>
<td>Sec Target [°C]</td>
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<td>-</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>Step Delay (Cycles)</td>
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<td>-</td>
<td>1</td>
<td>-</td>
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<tr>
<td>Acquisition Mode</td>
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<td>None</td>
<td>Single</td>
<td>None</td>
</tr>
</tbody>
</table>

(Melting not relevant for detection)

7.2. Data Analysis

For use in LightCycler® 1.x Instruments select channel F2 instead of channel 640 and channel F3 instead of channel 705 for detection.

Switch the color compensation mode on. If this mode is not enabled run the color compensation program. Follow the instructions in the manual of the Roche Diagnostics ‘LightCycler® – Color Compensation Kit’ (LightCycler® 1.x Instrument) / ‘LightCycler® Multicolor Demo Set’ (LightCycler® 2.0 Instrument).

Perform data analysis, as described in the LightCycler® Instrument operator’s manual.

We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user’s influence.

View Salmonella spp. data in channel 640, Quantification mode. The negative control (NTC) must show no signal.

If the internal control is used, view IC data in channel 705 Quantification mode. The negative control and the low-concentrated Salmonella spp. DNA samples (10 to 1,000 copies) should show an amplification curve for the IC with a CP at approximately cycle 31.

The provided standard row of cloned and purified DNA with concentrations in the range from $10^6$ copies/rxn to $10^7$ copies/rxn of Salmonella spp. should have CPs between cycles 17 and 35 (CPs calculated with Second Derivative Maximum method).
7.3. Sample Data – typical results

**Fig.1.** Sample data for the *Salmonella* spp. detection system.

**Upper panels:** Data from LightCycler® 2.0 Instrument. Left panel channel 640 quantification mode (Second Derivative Maximum) with standard curve for *Salmonella* spp. Right panel channel 640 melting analysis for *Salmonella* spp. (not relevant for detection).

**Lower panels:** Data from LightCycler® 2.0 Instrument. Left panel channel 705 quantification mode (Second Derivative Maximum) for the IC. Right panel channel 705 melting analysis for the IC (not relevant for detection).

7.4. Interpretation of data

<table>
<thead>
<tr>
<th><strong>Salmonella spp.</strong> (sample)</th>
<th><strong>IC (sample)</strong></th>
<th><strong>NTC</strong></th>
<th><strong>Result</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>no amplification detectable</td>
<td>negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>amplification signal</td>
<td>not relevant</td>
<td>negative</td>
<td>Positive</td>
</tr>
<tr>
<td>no amplification not detectable</td>
<td>not relevant</td>
<td>PCR failure, repeat experiment</td>
<td></td>
</tr>
<tr>
<td>amplification signal</td>
<td>not relevant</td>
<td>positive</td>
<td>Contamination, repeat experiment</td>
</tr>
</tbody>
</table>

Tab. 3. Typical analysis results
8. LightCycler® 480 II Instrument

8.1. Programming

The protocol consists of four program steps

1: Denaturation: sample denaturation and enzyme activation
2: Cycling: PCR-amplification of the target DNA
3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
4: Cooling: cooling the instrument

Detection Format:
LightCycler® 480 II Instrument: 498-640, 498-660

<table>
<thead>
<tr>
<th>Program Step</th>
<th>Denaturation</th>
<th>Cycling</th>
<th>Melting</th>
<th>Cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis Mode</td>
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<tr>
<td>Target [° C]</td>
<td>95</td>
<td>95</td>
<td>62</td>
<td>40</td>
</tr>
<tr>
<td>Hold [hh:mm:ss]</td>
<td>00:10:00</td>
<td>00:00:05</td>
<td>00:00:05</td>
<td>00:00:30</td>
</tr>
<tr>
<td>Ramp Rate [° C/s]</td>
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<td>Sec Target [° C]</td>
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<tr>
<td>Step Size [° C]</td>
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<td>0.5</td>
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<tr>
<td>Step Delay (Cycles)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acquisition Mode</td>
<td>None</td>
<td>None</td>
<td>Single</td>
<td>None</td>
</tr>
<tr>
<td>Acquisition [° C]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

(Melting not relevant for detection)

8.2. Data Analysis

Switch the color compensation mode on. If this mode is not enabled run the color compensation program. Follow the instructions in the manual of the Roche Diagnostics ‘LightCycler® Multicolor Demo Set’.

Perform data analysis, as described in the LightCycler® Instrument operator’s manual.
We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *Salmonella spp.* data with Filter Combination 498-640 Quantification mode. The negative control (NTC) must show no signal.

If the internal control is used, view IC data with Filter Combination 498-660, Quantification mode. The negative control and the low-concentrated *Salmonella spp.* DNA samples (10 to 1,000 copies) should show an amplification curve for the IC with a CP at approximately cycle 31.

The provided standard row of cloned and purified DNA with concentrations in the range from 10^6 copies/rxn to 10^7 copies/rxn of *Salmonella spp.* should have CPs between cycles 18 and 35 (CPs calculated with Second Derivative Maximum method).
8.3. Sample Data – typical results

![Sample Data Diagram]

**Fig. 1.** Sample data for the *Salmonella* spp. detection system.

**Upper panels:** Data from LightCycler® 480 II Instrument. Left panel Filter Combination 498-640 quantification mode (Second Derivative Maximum) with standard curve for *Salmonella* spp. Right panel Filter Combination 498-640 melting analysis peaks for *Salmonella* spp. (not relevant for detection).

**Lower panels:** Data from LightCycler® 480 II Instrument. Left panel Filter Combination 498-660 quantification mode (Second Derivative Maximum) for the IC. Right panel Filter Combination 498-660 melting analysis for the IC (not relevant for detection).

8.4. Interpretation of data

<table>
<thead>
<tr>
<th><em>Salmonella</em> spp. (sample)</th>
<th>IC (sample)</th>
<th>NTC</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>no amplification</td>
<td>detectable</td>
<td>negative</td>
<td>Negative</td>
</tr>
<tr>
<td>amplification signal</td>
<td>not relevant</td>
<td>negative</td>
<td>Positive</td>
</tr>
<tr>
<td>no amplification</td>
<td>not detectable</td>
<td>not relevant</td>
<td>PCR failure, repeat experiment</td>
</tr>
<tr>
<td>amplification signal</td>
<td>not relevant</td>
<td>positive</td>
<td>Contamination, repeat experiment</td>
</tr>
</tbody>
</table>

**Tab. 3.** Typical analysis results
9. Version history

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>V100830</td>
<td>Release version</td>
</tr>
<tr>
<td>V120323</td>
<td>Implementation of version history</td>
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