**Listeria monocytogenes Detection LyoKit Quick Guide**

**- 5'Nuclease -**

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PCR kit for the qualitative detection of *L. monocytogenes* DNA using real-time PCR instruments. Before starting, it is strongly recommended to read the entire product manual available on our website.

**PROGRAM SETUP**

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

- FAM (*L. monocytogenes*) and VIC (Internal Control).

As an alternative to VIC, HEX can be used. For the PikoReal® 24, Yakima Yellow has to be selected.

For some real-time PCR instruments the probe quencher as well as the usage of a passive reference dye has to be specified. This kit contains probes with a non-fluorescent “dark” quencher and no passive reference dye.

**DATA INTERPRETATION**

Verify results of positive (Control Template) and negative controls (H₂O), before interpreting sample results. Always compare samples to positive and negative control. Review data from each channel and interpret results as described in the table.

<table>
<thead>
<tr>
<th><strong>FAM</strong></th>
<th><strong>VIC</strong></th>
<th><strong>Result Interpretation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+ or -</td>
<td>Positive for <em>L. monocytogenes</em></td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>Negative for <em>L. monocytogenes</em></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

**Pre-incubation: 1 cycle**

- Step 1: 37 °C for 4 min
- Step 2: 95 °C for 5 min

**Amplification: 50 cycles**

- Step 1: 95 °C for 5 sec*
- Step 2**: 60 °C for 60 sec

* Please use 15 seconds for the LightCycler 96
** Fluorescence detection

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PREPARATION OF THE PCR MIX
Take appropriate precautions to prevent contamination, e.g. by using filter tips and wearing gloves.

1. PLACE STRIPS IN RACK
Take needed number of PCR tube strips out of aluminum bag. Important: close bag tight afterwards. Place strips in a suitable PCR tube rack. If needed, gently tap the tubes to move the lyophilized pellets to the bottom of all tubes.

2. DECAP
Open strips carefully direct before filling and discard caps. Do not leave open longer than necessary.

3. ADD SAMPLES AND CONTROLS
Pipet 25 µl of samples, negative control (colorless cap) or Control Template (purple cap) into respective wells. If using less volume, add PCR-grade H₂O to reach 25 µl.

4. SEAL
Seal the tubes with the provided 8-Cap Strips accurately.

5. MIX
Resuspend pellet after sealing by mixing thoroughly. Alternatively resuspend pellet by pipetting up and down multiple times in step 3.

6. CENTRIFUGE
Briefly spin strips, e.g. 5 sec at 500 - 1,000 x g, in a suitable centrifuge.

7. START REAL-TIME PCR RUN
Cycle samples as described above. Place tubes in a vertical, balanced order into the cycler, e.g. two strips can be placed in the first and last column.