**Quick Reference Procedure**

**- 5’Nuclease -**

Order no. R 302 20

PCR kit for the qualitative detection of *Listeria* genus 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 (Listeria sensu stricto)** 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 TAMRA as 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>FAM</th>
<th>VIC</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+ or -</td>
<td>Positive for <em>Listeria</em> sensu stricto</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>Negative for <em>Listeria</em> sensu stricto</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

* 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. Thaw reagents, mix (do not vortex!), and briefly spin vials before opening.

1. **PREPARE PCR MIX**
   Add 18 µl of Master Mix (yellow cap), 1 µl Enzyme Solution (red cap) and 1 µl Internal Control (white cap) for each reaction to a suitable tube (n samples + 2 controls + at least one additional reaction to cover pipetting loss). Mix carefully but thoroughly by pipetting up and down.

2. **ADD PCR MIX**
   Pipet 20 µl of prepared PCR mix into each strip or plate well.

3. **ADD SAMPLES AND CONTROLS**
   Pipet 5 µl of samples, negative control (colorless cap) or Control Template (purple cap) into respective wells.

4. **SEAL**
   Seal strips/plate accurately.

5. **CENTRIFUGE**
   Briefly spin strips/plate in a suitable centrifuge.

6. **START REAL-TIME PCR RUN**
   Cycle samples as described above.