**Listeria monocytogenes**

**Multiporator/Eppendorf Eporator®**

### Transformation Protocol

**Protocol No. 4308 915.520 – 12/2001**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Listeria monocytogenes 23074</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Bacteria, gram positive</td>
</tr>
<tr>
<td>Molecules injected</td>
<td>Plasmid DNA</td>
</tr>
<tr>
<td>Growth medium</td>
<td>BHI, supplemented with 0.5 M sucrose</td>
</tr>
<tr>
<td>Washing solution</td>
<td>1 mM HEPES (pH 7.0), 0.5 M sucrose</td>
</tr>
<tr>
<td>Electroporation solution</td>
<td>1 mM HEPES (pH 7.0), 0.5 M sucrose</td>
</tr>
<tr>
<td>Outgrowth medium</td>
<td>BHI, supplemented with 0.5 M sucrose</td>
</tr>
<tr>
<td>Cuvette</td>
<td>1 mm gap width</td>
</tr>
</tbody>
</table>

**Reference**

Park, S. and Stewart, G. • 1990 • Gene 94 • 129-132

### Making electrocompetent cells:

1. Dilute an overnight culture of *L. monocytogenes* 23074 in BHI, into fresh media (1:100). Grow at 37 °C with shaking until reaching an O.D.₆₀₀ of 0.2.
2. Add Penicillin G (10 µg/ml) and continue incubation for a further 2 hours.
3. Harvest by centrifugation (8,000 x g, 10 min., at 4 °C) and wash three times in ice-cold washing solution, once with equal volume and twice with ½ volume.
4. Resuspend the cells in ice-cold electroporation solution (0.0025 vol.). Use cells within 30 minutes of their preparation.

### Electroporation of cells:

1. Add 1 µg plasmid DNA to 100 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:
   - **Mode**: Prokaryotes “O”
   - **Voltage (V)**: 1,000 V
   - **Time constant (τ)**: 5 ms
4. Immediately add 1 ml of BHI medium and incubate at 37 °C for one hour.
5. Plate cells on selective BHI plates.

### Expected results:

Transformation efficiency up to 4 x 10⁶ transformants/µg of DNA.