Luminescent Bacteria Test (Microtox) with *Aliivibrio fischeri*

Bioassay with the marine luminescent bacterium *Aliivibrio fischeri* to detect a general toxicity resp. to detect substances with an inhibitory effect on bioluminescence.

**Test principle**
- Bioluminescence is linked to the cell metabolism.
- Toxic effects on the cell metabolism are shown by the inhibition of bioluminescence.

\[
\text{FMNH}_2 + O_2 + R-\text{CHO} \rightarrow \text{FMN} + H_2O + R-\text{COOH} + h\nu
\]

**Endpoint**
- Inhibition of bioluminescence [%] (general toxicity)

**Measurement with a luminometer at 0h**

Plate A: Test culture

- Merge plates A and B

**Incubation:**
- 30 minutes at room temperature in the dark

**Measurement with a luminometer after 30 minutes**

Plate B: Sample plate

**Measurement with a luminometer at 0h**

**Inhibition %**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>3.5-Dichlorophenol</th>
<th>3.5-Dichlorophenol</th>
<th>3.5-Dichlorophenol</th>
<th>Sample 1</th>
<th>Sample 1</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 2</th>
<th>Sample 2</th>
<th>Solvent control</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3%</td>
<td>99.8%</td>
<td>99.8%</td>
<td>99.9%</td>
<td>17.0%</td>
<td>20.7%</td>
<td>17.0%</td>
<td>93.9%</td>
<td>95.4%</td>
<td>95%</td>
<td>1.3%</td>
<td>-1.3%</td>
</tr>
<tr>
<td>-1.5%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>8.0%</td>
<td>6.6%</td>
<td>8.8%</td>
<td>80.0%</td>
<td>87.0%</td>
<td>82.5%</td>
<td>-1.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>-4.5%</td>
<td>98.0%</td>
<td>97.9%</td>
<td>97.8%</td>
<td>6.1%</td>
<td>1.3%</td>
<td>6.1%</td>
<td>56.9%</td>
<td>63.4%</td>
<td>59.8%</td>
<td>-4.5%</td>
<td>4.5%</td>
</tr>
<tr>
<td>-2.5%</td>
<td>53.4%</td>
<td>54.3%</td>
<td>54.7%</td>
<td>11.5%</td>
<td>9.9%</td>
<td>9.9%</td>
<td>33.7%</td>
<td>33.4%</td>
<td>32.2%</td>
<td>-2.5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>-0.8%</td>
<td>-3.3%</td>
<td>-16.0%</td>
<td>-10.1%</td>
<td>0.4%</td>
<td>3.3%</td>
<td>0.4%</td>
<td>21.4%</td>
<td>21.1%</td>
<td>16.1%</td>
<td>-0.8%</td>
<td>0.8%</td>
</tr>
<tr>
<td>1.4%</td>
<td>-13.2%</td>
<td>-9.4%</td>
<td>-17.7%</td>
<td>3.6%</td>
<td>5.2%</td>
<td>3.6%</td>
<td>10.6%</td>
<td>14.5%</td>
<td>8.1%</td>
<td>1.4%</td>
<td>-1.4%</td>
</tr>
<tr>
<td>2.2%</td>
<td>-10.6%</td>
<td>-7.0%</td>
<td>-14.1%</td>
<td>6.2%</td>
<td>6.2%</td>
<td>6.2%</td>
<td>9.6%</td>
<td>10.4%</td>
<td>14.2%</td>
<td>2.2%</td>
<td>-2.2%</td>
</tr>
<tr>
<td>-0.1%</td>
<td>-4.1%</td>
<td>18.8%</td>
<td>-20.3%</td>
<td>4.5%</td>
<td>-2.4%</td>
<td>4.5%</td>
<td>-1.1%</td>
<td>-5.4%</td>
<td>0.9%</td>
<td>-0.1%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

**Inhibition of bioluminescence (%)**

- 3.5-Dichlorophenol
- Solvent control
- Sample 1
- Sample 2

**Concentration (log) / dilution factor**

### Table

<table>
<thead>
<tr>
<th>Buffer</th>
<th>3.5-Dichlorophenol</th>
<th>3.5-Dichlorophenol</th>
<th>3.5-Dichlorophenol</th>
<th>Sample 1</th>
<th>Sample 1</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 2</th>
<th>Sample 2</th>
<th>Solvent control</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3%</td>
<td>99.8%</td>
<td>99.8%</td>
<td>99.9%</td>
<td>17.0%</td>
<td>20.7%</td>
<td>17.0%</td>
<td>93.9%</td>
<td>95.4%</td>
<td>95%</td>
<td>1.3%</td>
<td>-1.3%</td>
</tr>
<tr>
<td>-1.5%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>8.0%</td>
<td>6.6%</td>
<td>8.8%</td>
<td>80.0%</td>
<td>87.0%</td>
<td>82.5%</td>
<td>-1.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>-4.5%</td>
<td>98.0%</td>
<td>97.9%</td>
<td>97.8%</td>
<td>6.1%</td>
<td>1.3%</td>
<td>6.1%</td>
<td>56.9%</td>
<td>63.4%</td>
<td>59.8%</td>
<td>-4.5%</td>
<td>4.5%</td>
</tr>
<tr>
<td>-2.5%</td>
<td>53.4%</td>
<td>54.3%</td>
<td>54.7%</td>
<td>11.5%</td>
<td>9.9%</td>
<td>9.9%</td>
<td>33.7%</td>
<td>33.4%</td>
<td>32.2%</td>
<td>-2.5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>-0.8%</td>
<td>-3.3%</td>
<td>-16.0%</td>
<td>-10.1%</td>
<td>0.4%</td>
<td>3.3%</td>
<td>0.4%</td>
<td>21.4%</td>
<td>21.1%</td>
<td>16.1%</td>
<td>-0.8%</td>
<td>0.8%</td>
</tr>
<tr>
<td>1.4%</td>
<td>-13.2%</td>
<td>-9.4%</td>
<td>-17.7%</td>
<td>3.6%</td>
<td>5.2%</td>
<td>3.6%</td>
<td>10.6%</td>
<td>14.5%</td>
<td>8.1%</td>
<td>1.4%</td>
<td>-1.4%</td>
</tr>
<tr>
<td>2.2%</td>
<td>-10.6%</td>
<td>-7.0%</td>
<td>-14.1%</td>
<td>6.2%</td>
<td>6.2%</td>
<td>6.2%</td>
<td>9.6%</td>
<td>10.4%</td>
<td>14.2%</td>
<td>2.2%</td>
<td>-2.2%</td>
</tr>
<tr>
<td>-0.1%</td>
<td>-4.1%</td>
<td>18.8%</td>
<td>-20.3%</td>
<td>4.5%</td>
<td>-2.4%</td>
<td>4.5%</td>
<td>-1.1%</td>
<td>-5.4%</td>
<td>0.9%</td>
<td>-0.1%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

**Graph**

- **Inhibition of bioluminescence (%)**
- **Concentration (log) / dilution factor**

**Diagram**

- Reconstitution solution
- *Aliivibrio fischeri* (lyophilised)
- Incubation 15°C
- Measurement with a luminometer at 0h
- Plate A: Test culture
- Merge plates A and B
- Plate B: Sample plate
- Extracted environmental sample in solvent

**Diagram**

- Test principle
- Endpoint
- Measurement with a luminometer at 0h
- Incubation 15°C
- Plate A: Test culture
- Plate B: Sample plate
- Extracted environmental sample in solvent