**β-Galactosidase Assay for B. subtilis (Miller, 1972)**

**Example of culture preparation**

- Inoculate LB medium 1:100 with a fresh overnight culture carrying a promoter-lacZ-fusion and incubate on a shaker at 37°C
- At OD$_{600}$ 0.4-0.5 split the culture to 2 ml samples, induce one sample with e. g. an antibiotic, leave one sample as an uninduced control
- After 30 min, harvest cells by centrifugation and store the pellet at -20°C or continue directly with the assay

**β-Galactosidase Assay**

- Resuspend the cell pellet in 1 ml working buffer
- In a cuvette dilute the samples with working buffer until OD$_{600}$ is between 0.2 and 0.8 in a final volume of 800 µl (usually 500 µl working buffer and 300 µl of cells)
- Measure OD$_{600}$, use 800 µl working buffer as blank
- Add 10 Lysozyme, vortex and incubate at 37°C for 15-45 min, check if the sample is clear
- Add 150 µl ONPG, mix well and record time (=t₀)
- Incubate at room temperature until the sample turns yellow
- Stop the reaction by adding 400 µl Na$_2$CO$_3$, mix well and record time (=tₙ)
- If the samples do not turn yellow, stop the reaction after 60 min
- Measure OD$_{420}$ and OD$_{550}$ of each sample, use a cuvette with everything but the cells as blank
- Calculate promoter activity according to the formula:

$$Miller\ Units = \frac{1000 \times (A_{420} - (1.75 \times OD_{550}))}{(t \times v \times OD_{600})}$$

- A$_{420}$ absorption at 420 nm
- A$_{550}$ absorption at 550 nm
- t time of reaction (Tₙ - T₀)
- v volume of sample (usually 0.8 ml)
- A$_{600}$ absorption at 600 nm
Solutions

Lysozyme 15 mg/ml in Z-buffer
Na₂CO₃ 1 M
ONPG (2-nitrophenyl-β-D-galactopyranoside) 4 mg/ml in Z-buffer

Z-buffer (pH 7.0)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂HPO₄ * 2 H₂O</td>
<td>60 mM</td>
<td>10.68 g</td>
</tr>
<tr>
<td>NaH₂PO₄ * H₂O</td>
<td>40 mM</td>
<td>5.52 g</td>
</tr>
<tr>
<td>KCl</td>
<td>10 mM</td>
<td>0.75 g</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1 mM</td>
<td>0.24 g</td>
</tr>
<tr>
<td>H₂O</td>
<td></td>
<td>ad 1000 ml</td>
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</tbody>
</table>

Working buffer (prepare fresh) Z-buffer

20 mM β-Mercaptoethanol (139.5 µl to 100 ml Z-buffer)

Protocol generously provided by the lab
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