Preparing FACS samples
### Table of contents

Preparing FACS samples

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Preparing FACS samples</td>
<td>3</td>
</tr>
<tr>
<td>1.1</td>
<td>Materials</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Setup &amp; Protocols</td>
<td>3</td>
</tr>
</tbody>
</table>
## 1 Preparing FACS samples

**Estimated bench time:** 90 minutes  
**Estimated total time:** 3 hours

**Purpose:** Preparing the bacteria (after protein expression) for a FACS measurement. The bacteria will be extracted from the culture media and a fluorescent dye is added to covalently bind to the proteins.

### 1.1 Materials

- 1.5 ml cuvettes  
- 5 mM DBCO-PEG4-TAMRA  
- Cell Density Meter (OD600)  
- ddH2O  
- Eppendorf tubes  
- MiniSpin centrifuge  
- PBS-0.5%BSA  
- Pipettes and tips  
- Shaking block  
- Tabletop centrifuge  
- Tin foil

### 1.2 Setup & Protocols

- Spin down the cells in the tabletop centrifuge for 15 minutes at 3,000 xg and 4°C.  
- Discard supernatant.  
- Resuspend with 1 ml PBS-0.5%BSA and transfer to a 1.5 ml Eppendorf tube.  
- Spin down the cells in the MiniSpin centrifuge for 1 minute at 13,400 rpm.  
- Discard supernatant  
- Resuspend with 1 ml PBS-0.5%BSA.  
- Perform an OD measurement on a 20x dilution of the culture sample.  
  OD measurement (OD600):  
  - Blank: 950 μl ddH2O and 50 μl PBS-0.5%BSA  
  - Sample: 950 μl ddH2O and 50 μl PBS-0.5%BSA  
- Multiply the OD with 20.  
- Calculate the amount of cells in the culture using the Agilent Technologies website.  
- Make a dilution with a concentration of 1*10^9 cells/ml.  
- Prepare tubes for the FACS (mix well & cover the samples).

<table>
<thead>
<tr>
<th>Tube</th>
<th>[DBCO]</th>
<th>Cells [1*10^9] (μl)</th>
<th>DBCO-TAMRA (5mM) (μl)</th>
<th>Contains pAzf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>200</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>30 μM</td>
<td>200</td>
<td>1.21</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>30 μM</td>
<td>200</td>
<td>1.21</td>
<td>No</td>
</tr>
</tbody>
</table>

- Incubate the samples in a shaking block for 1 hour at 300 rpm and 4°C. Make sure the tubes are in the dark.  
- Spin down the cells in the MiniSpin centrifuge for 10 minutes at 13,400 rpm.

- Discard the supernatant.
- Resuspend with 1 ml ice-cold-PBS-0.5%BSA. (keep the tubes on ice)
- Spin down the cells for 10 minutes at 13,400 rpm.
- Discard the supernatant and put the pellet on ice in the dark until FACS.
- For the FACS: resuspend with 200 μl ice-cold PBS-0.5%BSA.