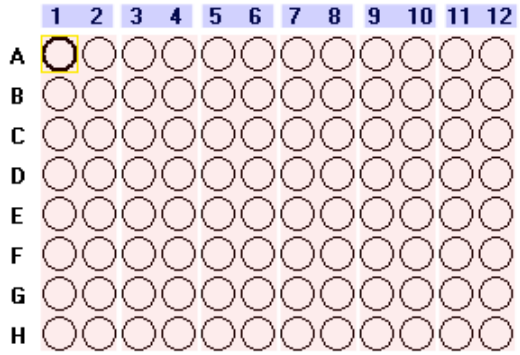
# Preparing a 96-well plate for HHL assay

## 1. Set-up




Column1: 180µl LB-medium + 20µl $H_2O$
Column2: 180µl cells + 20µl $H_2O$
Column3: 200µl cells in 0.01nM HHL
Column4: 200µl cells in 0.1nM HHL
Column5: 200µl cells in 1nM HHL
Column6: 200µl cells in 10nM HHL
Column7: 200µl cells in 50nM HHL
Column8: 200µl cells in 100nM HHL
Column9: 200µl cells in 200nM HHL
Column10: 200µl cells in 500nM HHL
Column11: 200µl cells in 1000nM HHL
Column12: 200µl cells in 2000nM HHL

## 2. Pipetting scheme

### 2.1. Stock solutions

| Final con. / µM | Stock used / µM | Vol. of stock / µl | Vol. of water / µl |
|---|---|---|---|
| 150 | 15000 | 12 | 1188 |
| 15 | 150 | 120 | 1080 |
| 1.5 | 15 | 120 | 1080 |
| 0.15 | 1.5 | 120 | 1080 |
| 0.015 | 0.15 | 120 | 1080 |

### 2.2. 94-well plate

The volumes below are sufficient for pipetting four well plates at a time.

| Final con. / nM | Stock used / µM | Vol. of stock / µl | Vol. of water / µl |
|---|---|---|---|
| 0.01 | 0.015 | 4.8 | 715.2 |
| 0.1 | 0.015 | 48 | 672 |
| 1 | 0.15 | 48 | 672 |
| 10 | 0.15 | 480 | 240 |
| 50 | 1.5 | 240 | 480 |
| 100 | 1.5 | 480 | 240 |
| 200 | 15 | 96 | 624 |
| 500 | 15 | 240 | 480 |
| 1000 | 15 | 480 | 240 |
| 2000 | 150 | 96 | 624 |

Pipet 20µl of the above concentrations into the corresponding wells and add 180µl of cell suspension right before the measurement of fluorescence.

## 3. Tecan plate reader

Start the machine 20 minutes in advance and heat the chamber to 37°C. Put in the plate and start the measurement with the following setting:

- Measure OD at 612 nm
- Measure fluorescence with an excitation wavelength of 485nm and an emission wavelength of 485nm for both GFP and YFP
- Shake
- Repeat measurements every 5min for 3 hours

## 4. Data processing

- Normalize the data by dividing all fluorescence data by the corresponding optical density
- Subtract the obtained value of the reference column (column 2) from the calculated relative fluorescence of the wells containing HHL
- Plot the fluorescence data over the time