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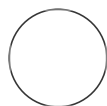
# Fluorescence\_activity\_assay\_Interlab\_Study\_PCC\_6803

In 1 collection

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 We use this protocol and it's working

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## ABSTRACT

Fluorescence activity assay for Synechococcus PCC 6803 strains during the interlaboratory study published by Mager et al. 2023.

## Preculture conditions of the fluorescence activity assay

- 1 Precultures were started from cultures derived from cryoconserved cells after 48h of growth in

copper free BG11-PC medium (hereafter referred to as BG11 medium).

#### Note

Please refer to our protocol "Cryo\_conservation\_Synechocystis\_PCC\_6803" for more details on growing PCC 6803 from cryo conserved cells and our protocol "BG11\_and\_inducer\_preparation" for the preparation of copper free BG11-PC medium

4 Strains were used for the fluorescence activity assay in the Interlab study. All strains were Synechocystis PCC 6803 mutants carrying a fluorescence reporter gene.

#### Note

Please refer to our manuscript for more details on the plasmids. plasmid maps can be found in our figshare repository under .....

- 1.1 Dilute all strains to an OD730 of 0.3 in 35ml of BG11 final volume in a 100ml Erlenmeyer flasks with a cotton plug

#### Note

Supply 10  $\mu\text{g mL}^{-1}$  chloramphenicol, which is added individually to each flask before inoculation.

- 1.2 Grow all cultures in shaking incubators set at 100 rpm under 50  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  constant white light illumination, ambient  $\text{CO}_2$ , and 30°C over night until OD730 0.5-0.6

## Preparing main cultures

- 2 Transfer all cultures into 50ml falcons



#### Note

Keep the flasks steril !

**2.1** Adjust the OD730 of all cultures to 0.5 with BG11 containing 10 µg mL<sup>-1</sup> chloramphenicol to a final volume of 50 ml

**2.2** Measure the full 400-750 nm OD730 spectrum and OD730 nm of these cultures



**Note**

This is your timepoint 0h value for the absorption spectrum. Refer to the last section of this manuscript for details on OD measurements.

**2.3** Rinse the preculture flasks with 25 ml Copper-free BG11 **twice**



**Note**

This is used to remove any residual copper. These flasks will be used for the uninduced cultures

**2.4** Fill 20 ml of each preculture adjusted to an OD730 of 0.5 back into the preculture flasks

**Note**

These will be your uninduced cultures !

**2.5** Fill 20 ml of each preculture adjusted to an OD730 of 0.5 into new 100ml erlenmeyer flasks with cotton plugs

**Note**

These will be your induced cultures !

**2.6** Add the respective inducer to the flasks prepared in Step 2.5  
To each Synechocystis strain carrying the following plasmid add 200 µl of the following inducer:

A	B
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A	B
strain	inducer
EVC	MilliQ water
prha_mVENUS	1M rhamnose
petE_mVENUS	100µM CuSo4
J23100_mVENUS	MilliQ water

List of strains and respective inducers used in the Interlab Study

#### Note

For the preparation of the inducers please refer to our protocol "BG11\_and\_inducer\_preparation".

**2.7** Put all flasks in a shaking incubators set at 100 rpm under 50 µmol photons · m<sup>-2</sup> · s<sup>-1</sup> constant white light illumination, ambient CO<sub>2</sub>, and 30°C

**2.8** From each culture, take 1 ml sample at timepoint 0, 2, 4, 5, 6, 7 and 24h starting from the addition of the inducers and use this sample to perform the OD730 and fluorescence intensity measurements described in the following two section immediately after sampling



## OD730 measurements in the spectrophotometer

**3** Dilute 500 µl of your sample with 500µl of BG11 in a spectrophotometer cuvette

#### Note

For the sample at timepoint 24h, instead dilute 200µl in 800µl of BG11.  
All measured samples should only be measured in the assumed linear range of OD730 from 0.1-0.5.

**3.1** Use 1 ml of BG11 to blank your spectrophotometer

### 3.2 Measure the absorption at 730nm

#### Note

For all samples at timepoint 0h and 7h, additionally measure the full 400-750 nm OD spectrum and of these cultures. This is your timepoint 0h and 7h value for the absorption spectrum.

## Fluorescence- and OD730 measurements in the plate reader

### 4 For each strain, fill 3 wells of a black, flat-bottomed 96 well plate with 100 µl of your sample

#### 4.1 Fill 4 wells with BG11 as blanks

#### 4.2 Measure the absorbance of each well at 730nm

#### 4.3 Measure fluorescence of each well with an excitation of 511 nm/ 12 nm bandwidth and an emission of 552 nm/ 20 nm bandwidth