APR 26, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.8epv5jdw5l1b/v1

Protocol Citation: maurice.mager1808 2023. Fluorescence_activity_assay_Interlab_Study_PCC_6803. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5jdw5l1b/v1

Protocol status: Working
We use this protocol and it's working

Created: Feb 17, 2023

Last Modified: Apr 26, 2023

PROTOCOL integer ID: 77177

Fluorescence_activity_assay_Interlab_Study_PCC_6803

In 1 collection

maurice.mager1808[1]

[1]HHU

Axmann Lab

maurice.mager1808

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 flaks with a cotton plug

Note

Supply 10 µg 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 µmol photons · m-2 · s-1 constant white light illumination, ambient 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-1 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

	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-2 · s-1 constant white light illumination, ambient CO_2, 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 spectrophotomer

3 Dilute 500 µl of your sample with 500µl of BG11 in a spectrophometer 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