

Growth curve for *Chlamydomonas reinhardtii*

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ABSTRACT

This protocols describe the steps required for obtain a growth curve of *Chlamydomonas reinhardtii* and fluorescent protein expression of mVenus and mCherry.

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KEYWORDS

Growth curve, *Chlamydomonas*, Absorbance, 96 well plate

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ABSTRACT


This protocols describe the steps required for obtain a growth curve of *Chlamydomonas reinhardtii* and fluorescent protein expression of mVenus and mCherry.

Material

- 1
 - TAP media or other (How to prepare TAP media [here](#))
 - Erlenmeyer flask
 - Orbital shaker
 - Light source
 - 96 well plate, Black Frame, Clear bottom (Ex:

Plate reader Settings

2 Reading are performed in a Black 96 well plate with clear bottom.

Absorbance set to  750 nm .






Fluorescence set as the Table below.







	Excitation (nm)	Emission (nm)	Gain	Optics position
Chlorophyll	440	680	70	bottom
mVenus	500	530	120	bottom
mCherry	583	613	150	bottom

Inoculum

2w 5d

2w 5d

3 Inoculate  1 mL of the cells from a culture in stationary phase ( 120:00:00 to  168:00:00) in an  100 mL erlenmeyer flask containing  50 mL of TAP media.

1. Place the flask in a  150 rpm, 25°C, 1cm of orbit with  80 µmol/m2s of incident white light ( 60 µmol/m2s to  120 µmol/m2s works)
2. Take a initial sample  100 µl of culture and measure it in the plate reader according to the settings above.
3. During  168:00:00 take samples at least once a day.
4. The final culture can be used for further test, as dry cell weight (DCW) determination.

Frequent sampling increase data quality, but it is advice to not remove more than 10% of culture in sampling during the entire procedure. Technical replicates are advice for each time point.
All culturing conditions are set initially, and can be change accordingly to the experiment goal.

Example of DCW protocol.



Dry cell weight by centrifugation
by Joao Vitor Molino,
University of Zürich

PREVIEW

RUN



- 3.1
- Analytical balance with high precision (*The higher the precision the better. For example a balance with a 0.1mg readability, could account to approximately 10% error alone in a measurement of 1mL sample of a culture at 1g/L*)
 - Microcentrifugal tubes
 - Microcentrifuge

1. Label microcentrifugal tubes

- 3.2
2. Dry the tubes at **90 °C** , **Overnight**
 3. Cool tubes at **Room temperature** for **00:30:00**
 4. Record the weight of the tubes
- 3.3
1. Harvest 2 mL of culture in a previously weighted tube
 2. Centrifuge the sample at **20000 rcf, 25°C, 00:01:00**
 3. Carefully remove the supernatant by pipetting
 4. Wash the cells with ddH₂O, and centrifuge the sample at **20000 rcf, 25°C, 00:01:00**
 5. Carefully remove the supernatant by pipetting
 6. Dry the tubes at **90 °C** , **Overnight**
 7. Cool tubes at **Room temperature** for **00:30:00**
 8. Record the weight of the tubes
 9. Subtract the initial tube weight to achieve the dry cell weight