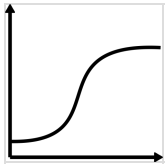

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Growth curve for *Chlamydomonas reinhardtii*

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1 *Works for me* This protocol is published without a DOI.

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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.

PROTOCOL CITATION

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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:

IsoPlateTM - 96F (Black Frame & Clear well 96-well) **Perkin Elmer Catalog #6005020**)

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

Inocullum 2w 5d

2w 5d

3 Innoculate **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,
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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 ddH20, 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