

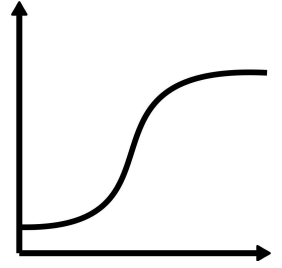


Nov 19, 2020

Growth curve for *Chlamydomonas reinhardtii*

DOI

dx.doi.org/10.17504/protocols.io.bpvbmn2n



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DOI: <https://dx.doi.org/10.17504/protocols.io.bpvbmn2n>

Protocol Citation: Joao Vitor Molino 2020. Growth curve for *Chlamydomonas reinhardtii*. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bpvbmn2n>

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Protocol status: Working

We use this protocol and it's working

Created: November 19, 2020

Last Modified: November 19, 2020

Protocol Integer ID: 44675

Keywords: Growth curve, Chlamydomonas, Absorbance, 96 well plate, growth curve of chlamydomonas reinhardtii, chlamydomonas reinhardtii, chlamydomona, fluorescent protein expression of mvenus, fluorescent protein expression, growth curve, fluorescent,

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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 materials

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

Troubleshooting



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:



IsoPlate™ - 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	Opti cs posi tion
	Chloroph yll	440	680	70	bott om
	mVenus	500	530	120	bott om
	mCherry	583	613	150	bott om

Inoculum

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.

2w 5d



1. Place the flask in a 150 rpm, 25°C ,1cm of orbit with 80 $\mu\text{mol}/\text{m}^2\text{s}$ of incident white light (60 $\mu\text{mol}/\text{m}^2\text{s}$ to 120 $\mu\text{mol}/\text{m}^2\text{s}$ 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.

Note

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.

Protocol



NAME

Dry cell weight by centrifugation

CREATED BY

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Preview

- 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
- 3.2
 1. Label microcentrifugal tubes
 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