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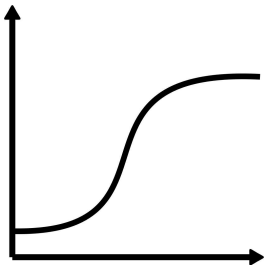
Growth curve for *Chlamydomonas reinhardtii*



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

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Disclaimer

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

IsoPlateTM - 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:

  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

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. 2w 5d

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.

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

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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 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