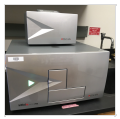

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Using the Tecan Infinite M200 Pro plate reader for Symbiodiniaceae Biomass/Growth Estimates

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1 Works for me dx.doi.org/10.17504/protocols.io.bb7girjw

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ABSTRACT

This is a protocol for estimating Symbiodiniaceae biomass and growth based on fluorescence (chlorphyll-a). This is not directly comparable to cell counts, as fluorescence might vary depending on culturing conditions.

GUIDELINES

Symbiodiniaceae are relatively heavy cells that sink quickly. Prepare no more than 2-3 samples at a time so you have enough time to mix and read the sample before they sink out, thus affecting fluorescence measurements.

MATERIALS

NAME	CATALOG #	VENDOR
Nunc™ MicroWell™ 96-Well Optical-Bottom Plates with Polymer Base, black	265301	Thermo Fisher

MATERIALS TEXT

You can use a different plate, but be sure to use the same one throughout your experiment and make sure it is compatible with the machine.

BEFORE STARTING

Make sure the Tecan is turned on and has warmed up for at least ~15 minutes.

1 Turn the Tecan Infinite M200 Pro plate reader on. There is a switch on the back of the big plate reader box, as well as on the smaller box on top. You should see a green light turn on or electronic dialogue pop up, letting you know both components are on.

2 On the desktop of the computer, click the "i-control 1.11 (for infinite reader)" software icon. It might take a while for this to load, but when it has, you should see a 96 well plate schematic that is highlighted in yellow.

2.1 You can set up/sterilize the space you are going to be sampling your cultures in during this time.

3 Once the software has loaded, select 2 or 3 wells that you wish to measure by clicking and dragging across the schematic on the software. Only these wells should be highlighted yellow now.

4 On the left side bar, double-click 'Fluorescence intensity". A new section should appear under the 96-well plate schematic where you can specify the excitation and emission wavelengths you want to use. All other default settings can remain unchanged.

4.1 For Symbiodiniaceae, we excite with blue light (488nm) and measure red fluorescence emissions (685nm). This excitation is also a commonly used wavelength for flow cytometer lasers, etc.

4.2 Other Default Settings:

Make sure 'Plate with cover' is UNCHECKED at the top.
Mode: Top
Z-Position: Manual 20,000 microns
Number of Flashes: 25
Gain: Manual 100
Integration time: 20 microseconds
**You can take multople reads per well if you want to take the average.

5 

Retrieve the cultures you wish to measure from the incubator and place these in your sterile working area. Using aseptic technique, homogenize the sample by pipetting up and down with a 1000 microliter pipette ~20 times. Observe the bottom of the flask to make sure no cells are still stuck to the bottom before proceeding.

5.1 Note: Symbiodiniaceae are typically not axenic cultures. Some literature has demonstrated that contaminants can cause Symbiodinium to develop 'microbialte' formations which are a mixture of calcium carbonate precipitants and potentially biofilms (Frommlet et al. 2014 PNAS). Keeping the cultures mixed seems to prevent microbialte formation, and in our hands we have achieved this by pipette mixing every day. The longer the cultures go undistrubed, the harder it is to dislodge biomass from the culture flask walls/bottom, which potentially affects both growth measurements and experiments.

6 Once the culture is well mixed, collect 250 microliters and transfer it to a designated well in the 96-well plate. Make sure there are no bubbles in the sample.

7 Open the Tecan Infinite M200 Pro door by pushing the triangular button on top of the plate reader box. The door should automatically open, allowing you to set your tray inside. Press the button again so the tray will retract into the machine and close the door.

7.1 Make sure to remove the plate lid before inserting your plate.

8 If all your settings are correct, click the "Start" button at the top of the software screen.

8.1 An excel sheet should automatically appear with your instrument settings and well sample measurements, which will appear in real time. Save the data in the Correa Lab directory. If you have more culture samples to measure, you can leave the excel sheet open and a new tab will be added for each additionaly measurement. Otherwise you can close the excel sheet and have the software generate a new excel document for each sample.

9 Upload all your data for that day to the cloud (i.e. Samantha's shared OneDrive folder). Organize the file systems by growth curve/project and date, and keep a summary account of your daily measurements for each culture in a master excel sheet and/or your lab notebook. This will be used to create growth curve figures and calculate growth rates during logarithmic phase.