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Reading Sample Fluorescence (PDMPO) via Fluorometer

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EXTERNAL LINK

http://wilhelmlab.utk.edu/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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GUIDELINES

Fluorometer Specifications: In this procedure, we utilize a Turner Designs-700 Laboratory fluorometer equipped with Andover Corporation 530 nm filter and 370 nm filter for detecting PDMPO fluorescence (excitation wavelengths 360-380nm, emission wavelengths 522-542nm).

Establishing a Standard Curve: Prior to ***centrifuging samples** for reading, establish a standard curve of 5 different concentrations (standard curve replicates are to be: 4mL NaOH that has been incubated at 80°C in the hot water bath along with the other samples, chilled on ice and neutralized with + 1mL 1M HCl, with selected volume of PDMPO then added prior to reading). Also ensure there is a blank, consisting of only the NaOH-HCl matrix with no PDMPO addition.

Considerations:

-The highest standard should be approximately 80% of your max range entered.

-The difference between standards should not be less than 10% of the entered maximum range.

-When forming standard curve, it is helpful to take into account the initial volume of PDMPO added when designating the highest standard (Standard 5). For example, if inoculating samples with 15mL PDMPO, then there will only be at most 15mL of PDMPO in your digestion matrix, making the over-estimation that the diatoms somehow took all the PDMPO dye up and incorporated it after your time series. Selecting a linear number of concentrations scaling down from 15mL will allow you to establish a standard curve likely to have a high R^2 value.

MATERIALS TEXT

Utilize a TD-700 Laboratory Fluorometer linked to a computer monitor with the downloaded Turner software to obtain fluorescence readings of the samples

SAFETY WARNINGS

See SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

Establishing a Standard Curve: Prior to centrifuging samples for reading, establish a standard curve of 5 different concentrations. Standard curve replicates are to be:

- **4 ml NaOH** that has been incubated at **80 °C** in the hot water bath along with the other samples.

- Chilled on ice and neutralized with + **1 ml** **1 Molarity (M) HCl** with selected volume of PDMPO then added prior to reading.

- Ensure there is a blank, consisting only of the NaOH-HCl matrix with no PDMPO addition.

Calibration

1 From the home screen of the device, perform the following:

1.1 Select the 1. Button for “set-up”

1.2 Select the 1. Button again for “mode”

2 Confirm machine reads “simple multi-operational” (which is the mode)

press ENTER.

3 Select the 2. Button for “calibration procedure”

3.1 Use < > arrow buttons to select “Direct concentration” (as the type of measurement to be taken/read)

press ENTER.

4 Select the 3. Button for “units”

Then select mg/L, mg/mL, ng/mL etc.

Depending on your own concentrations selected for your standards. We utilized mg/mL or ng/mL for our standards, depending on whether they are cultures or environmental samples.

5 **Press ESCAPE** and return to the main home screen.

6 Select “max range”

Which is the maximum approximate concentration range you expect, you will have to perform a little trial and error depending on your samples and their concentration.

*This will vary on your cell concentration, incubation conditions, and the volume filtered.

7 You will then be prompted to enter the number of standards you are using (5) and **press ENTER**.

8 

The machine will ask you to insert your highest standard first:

Pipet **4 ml** of the standard into a disposable glass 10 mL tube and wiping the outside prior to insertion.

9 Enter the concentration (in your prior selected units) from your standard. (This is your highest standard).

Select 1. “OK”. Then **press “*”** to read the standard.

10 Machine will prompt you to repeat the same steps for your other standards (Order does not matter).

11 Follow prompt to insert a blank, press **ENTER**.

4 ml of NaOH and **1 ml** 1M HCl

12 Once blank stabilizes, **press "0".**

13 Screen will revert to home screen when reading is complete.

14 Once calibration is complete and displayed on the computer screen that is hooked up to your fluorometer,

You will use this equation to convert your sample readings (FSU) to actual concentrations of PDMPO and then silica.

It is recommended to record your fluorescence readings (FSU) of your standards as you go through the calibration process and plot them manually to validate and compare the program’s results (actual concentration, x-axis) vs. (fluorescence, y-axis).

15 The linear regression equation must have an R^2 value higher than 0.95 ($R^2 >$ 0.99 preferred).

Reading Samples

16 

Carefully pipette the top **4 ml** of matrix from your recently centrifuged samples into a clean 10 mL disposable glass tube.

Do not to disturb the bottom of the 15 mL Falcon tube where filter fragments and unlysed/digested cells may be, as it will interfere with fluorescence readings.

17 **Press the "*"** button and begin sample reading which will be visible on computer screen.

The end number is an average of a variety of fluorescence measurements.

18 After reading all samples, enter the fluorescence readings into your linear regression equation (y) and solve for the actual concentration of PDMPO (x).

The actual concentration must be converted from **mL/nL** to **mol.** Then converted to silica using the conversion factor 3230:1 for Si: PDMPO (mol:mol).

19 Normalize to either **cells/mL** or **chlorophyll-A** data, for each sample, to obtain Si (mol)/cell.

20 Information can be graphed to show silica deposition rates over time.