1. Procedure summary

Method for obtaining the fluorescence values of samples.

1.1. Related Procedures

Measuring OD of Samples in a 96-well Plate Using the Spectramax Plate Reader
Use of the Spectamax Plate Reader for Endpoint Measurements

1.2. Procedure impacts and concerns

Safety	Gloves should be worn at all times while performing this procedure.
Quality	Measurements are taken in triplicate and the variation between samples should be less than 20% RSD.
Delivery	Outside operations may need to change based on fluorescence data so it should be reported by 0900 hours.
Environmental	Local policies and procedures should be followed as determined by the site leadership.
Cost	Consumables- $0.45/sample Labor- 0.02 hours/sample
Compliance	Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required (see 29 CFR 1910.120 and 1200).

1.3. Responsibilities and owners

Document Owner	Manage content and distribution	Kari Mikkelson
Process Owner	Responsible for content and process validation	Rebecca White
Site Manager	Responsible for implementation and conformance	Rebecca White

2. Process

2.1. Process description

Fluorescence can be used as a way to quantify the amount of algae present in a given sample. Algae have pigmented compounds that will emit a fluorescent signal when excited at a specific wavelength. The intensity of this signal is proportional to the amount of pigment in the sample and can thus be used as a way to determine the concentration of algae present. This assay is carried out by diluting samples 10-fold in a 96-well plate and measuring the fluorescent signal emitted at different excitation wavelengths using the SpectraMax M2 plate reader. Four excitation-emission wavelengths are reported: Ex430/Em685, Ex450/Em685, Ex363/Em685, Ex590/Em650.

2.2. Process diagram: Work Instruction

Not applicable for this procedure.

Equipment and Supplies

96 –well culture plates (Fisher Scientific, Catalog# 08-772-2C)
20-200uL Single Channel Pipette
20-200uL Multichannel Pipette
2-20uL Multichannel Pipette
2-20uL Pipet Tips (Rainin, Catalog# SS-L10S)
20-250uL Pipet Tips (Rainin, Catalog# SS-L250S)
Gibco Ultrapure Water (dH_2O) (Life Technologies, Catalog# 10977-023)
Troughs (Fisher Scientific, Catalog# 07-200-127)
Eppendorf MixMate
SpectraMax M2 Plate Reader
Purple plate adapter
Pond samples

2.3. Process steps

2.3.1. Setting-up the Spectramax Plate Reader and Softmax Pro Software

2.3.1.1. Log onto the computer connected to the Spectramax and open the Softmax Pro software. The plate reader drawer will open.

2.3.1.2. From the Softmax Pro software open the Fluorescence protocol file. (L:\QAQC\SpectraMax Templates\Fluorescence Template)

2.3.1.3. Place the purple adapter into the plate drawer.

2.3.2. Sample Preparation

2.3.2.1. Arrange pond samples in number order from smallest to largest.

2.3.2.2. Pour about 20mL of dH_2O into a trough.

2.3.2.3. Using a single channel pipette aliquot 200uL of dH_2O into well A1 of a 96-well culture plate to use as a blank. (See Figure 1)

2.3.2.4. Using a multichannel pipette aliquot 180uL of dH_2O into the same 96-well plate according to the plate map below based on the number of pond samples to be analyzed. (See Figure 1)

2.3.2.5. Set a multichannel pipette to collect 20uL and affix it with 3 pipet tips.

2.3.2.6. Invert the bottle containing sample 1 several times to mix.

2.3.2.7. Using the multichannel pipette, aliquot 20uL from sample 1 into wells B1, C1, and D1.

2.3.2.8. Repeat steps 2.3.2.5-2.3.2.7 for all remaining samples using Figure 1 as a guide.

2.3.2.9. After all samples have been added to the 96-well plate place it in the Eppendorf MixMate and mix using the pre-programmed 96-well plate setting or pipet all wells up and down several times with a multichannel pipette.

<The lid for the 96-well plate must be off while mixing and reading the plate in the Spectramax.>

2.3.3. Reading the Plate & Data Analysis

2.3.3.1. After samples have been mixed, place the plate on top of the purple adapter in the plate reader drawer.

2.3.3.2. Press the “Read” button in the Softmax Pro software. The drawer will be retracted into the plate reader and data collection will begin.

2.3.3.3. When the read is complete the drawer will open again and the data will be displayed in the plate map of the Softmax Pro software.

2.3.3.4. Save the data in the QAQC folder on the Columbus drive.

2.3.3.5. Analyze the data using the Fluorescence Data Template. (L:\QAQC\Data Analysis Templates\Fluorescence Data Template)

<The Spectramax will make chirping noises while it is reading data from the plate and will take several minutes to complete data collection.>

2.3.3.6. Save the data analysis in the QAQC folder on the Columbus drive.
2.3.3.7. Repeat all above process steps for any samples that do not pass QC.
2.3.4. Clean-up and Spectramax Shut-Down
2.3.4.1. Remove the plate from the drawer and replace the lid. Dispose of the plate in an approved waste container.
2.3.4.2. Close the Softmax Pro software and log off the computer.
2.3.4.3. Remove the plate adapter and close the plate reader drawer by pressing the "Drawer" button on the bottom right of the Spectramax instrument panel.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank (dH2O)											
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
E												
F	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24
G	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24
H	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24

Figure 1. Plate map

3. Required documents

Input documents
Cultivation Daily Data Sheet

Output documents
Fluorescence Data Template — L:\QAQC\Data Analysis Templates\Fluorescence Data Template

4. Document control

Revision history

R0 – Initial Release – Nicole Heaps	<Date>
R1 – Aida Brooks	<Date>

Document approval
<Name> — <Approval date>

Document reviewers

Aida Brooks		04/2013
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5. Risk analysis

<Risk name>	<Mitigation plan>	<Owner>	<RPN>