1. Procedure summary

Method for obtaining the optical density (OD) of samples.

1.1. Related Procedures

Measuring Fluorescence of Samples in a 96-well Plate Using the Spectramax Plate Reader
Use of the Spectramax 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 OD 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- $0.37/sample Labor- 0.01 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

Absorbance or optical density (OD) is a ratio of light entering a sample, to the light that passed through the sample.This assay is carried out by performing an endpoint absorbance measurement. Samples are aliquoted into a 96-well plate and the absorbance is measured at two different wavelengths, 560nm and 750nm, using the SpectraMax M2 plate reader. The two wavelengths are used to evaluate biomass density of different types of algae. OD560 is used for cyanobacterial strains whereas OD750 is used for green algae.

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
20-250uL StableStak Pipet Tips (Rainin, Catalog# SS-L250S)
Gibco Ultrapure Water (dH2O) (Life Technologies, Catalog# 10977-023)
Eppendorf MixMate
SpectraMax M2 Plate Reader
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 OD&ODAC protocol file. (L:\QAQC\SpectraMax Templates\OD&ODAC Template)

2.3.2. Sample Preparation

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

2.3.2.2. 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)

2.3.2.3. Set a multichannel pipette to collect 200uL and affix it with 3 pipet tips.

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

2.3.2.5. Using the multichannel pipette aliquot 200uL from sample 1 into wells B1, C1, and D1 of the 96-well plate.

2.3.2.6. Repeat steps 2.3.2.3-2.3.2.5 for all remaining samples using Figure 1 as a guide.

2.3.2.7. 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 in the plate reader drawer of the Spectramax.

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 OD Data Template. (L:\QAQC\Data Analysis Templates\OD Data Template)

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.

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

2.3.4. Clean-up and Spectramax Shut-Down

2.3.4.1. Remove 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. 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	Samp	Samp	Samp	Samp	Samp	Samp	Samp	Samp	Samp	Samp	Samp	Samp

	le 1	le 2	le 3	le 4	le 5	le 6	le 7	le 8	le 9	le 10	le 11	le 12
E												
F	Samp le 13	Samp le 14	Samp le 15	Samp le 16	Samp le 17	Samp le 18	Samp le 19	Samp le 20	Samp le 21	Samp le 22	Samp le 23	Samp le 24
G	Samp le 13	Samp le 14	Samp le 15	Samp le 16	Samp le 17	Samp le 18	Samp le 19	Samp le 20	Samp le 21	Samp le 22	Samp le 23	Samp le 24
H	Samp le 13	Samp le 14	Samp le 15	Samp le 16	Samp le 17	Samp le 18	Samp le 19	Samp le 20	Samp le 21	Samp le 22	Samp le 23	Samp le 24

Figure 1. Plate map

3. Required documents

Input documents
Cultivation Daily Data Sheet

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

4. Document control

Revision history

R0 – Initial Release – Nicole Heaps	<Date>
R1 – Cheng Fang	04/2013

Document approval
<Name> — <Approval date>

Document reviewers
Cheng Fang — 12/2014
Aida Brooks
Kari Mikkelson

5. Risk analysis

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