

## 1. Procedure summary

Procedure for obtaining the dark-adapted photosynthetic yield measurements (PAM) of a culture.

### 1.1. Related Procedures

<Related procedure name>

<Related procedure number>

<Related procedure name>

<Related procedure number>

### 1.2. Procedure impacts and concerns

Safety

Pond samples may contain pesticides or other harmful chemicals, therefore gloves should be worn when working with samples.

<Additional notes>

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).

Quality

PAM measurements are taken in triplicate and should be within a 10%RSD.

<Additional notes>

Delivery

<Delivery impacts>

<Additional notes>

Environmental

<Environmental impacts>

<Additional notes>

Cost

<Cost impacts>

<Additional notes>

Compliance

<Compliance impacts>

<Additional notes>

### 1.3. Responsibilities and owners

Document Owner

Manage content and distribution

Cheng Fang

Process Owner

Responsible for content and process validation

Kari Mikkelsen

Site Manager

Responsible for implementation and conformance

Rebecca White

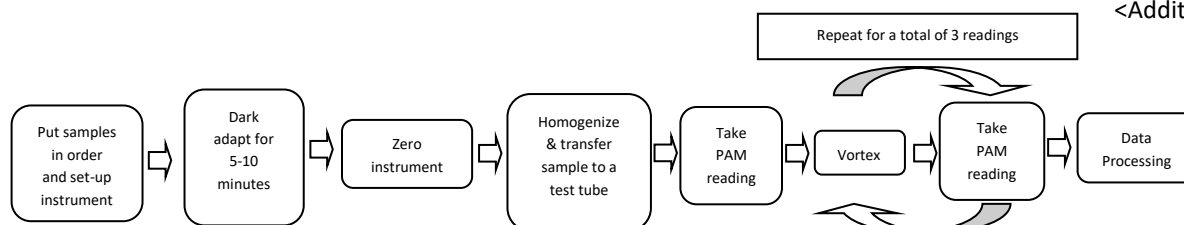
## 2. Process

### 2.1. Process description

PAM is a method used to measure the photosynthetic health of a culture. A sample is dark-adapted for 10-15 minutes and using a Walz MINI-PAM apparatus a short pulse of actinic light is applied to the sample. The apparatus gives a reading that is a ratio of the variable fluorescence/maximal fluorescence ( $F_v/F_m$ ) or photosynthetic yield.

<Additional notes>

### 2.2. Process diagram: Work Instruction



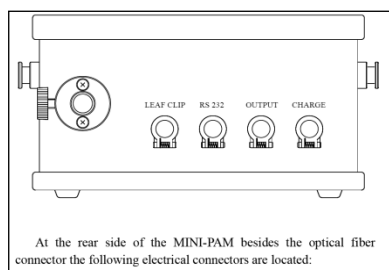
<Additional notes>

### Equipment and Supplies

Kimax Test Tube (Fisher Scientific, Catalog#S30531)  
Plastic Funnel  
Vortex  
PVC Plastic Stand  
Squirt Bottle with RO water  
Paper Towels  
Non-Hazardous Waste Container  
Walz MINI-PAM  
Computer with Wincontrol Software

### 2.3. Process steps

1. Put pond samples in order, so they can be easily measured in the dark.
2. Check that the RS232 cable is connected to the port on top of the PAM instrument. (Figure 1)
3. Log onto the computer and open the Wincontrol software program. Click on the Report tab.
4. Place a clean Kimax test tube into the PVC plastic stand. (Figure 2)
5. Fill the test tube with ~30mL of RO water. This will be your blank.
6. Insert the fiber optic light source into the opening on the side of the stand until it is pressed up against the test tube. (Figure 2)



**Figure 1. PAM electrical connectors**



**Figure 2. PVC plastic stand**

7. Cover the computer screen with a towel or shirt.
8. Place the pond samples in the cabinet and close the doors. Turn on the red light. Turn the lights off in the dark room and close the door. Allow the samples to dark adapt for at least 2 minutes.
9. After the samples have dark adapted, return to the room. Ensure the fiber optic light source is pressed up against the test tube containing the water.
10. Press the WinControl Zeroing Icon in upper right corner of the computer screen to blank the instrument. (Figure 3)
11. Press the SAT-Pulse icon (Figure 4) to record the zero value. Check that the  $F$ ,  $F_m'$ , and Yield values all read 0. If they do not repeat steps 2 and 3 until the values read 0.



**Figure 3. WinControl Zeroing Icon**

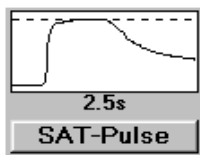


Figure 5. SAT-Pulse Icon

12. Dump the water into a waste container and place the test tube back in the PVC plastic stand. Place the funnel on top of the test tube.
13. Place your mouse over the SAT-Pulse icon.
14. Cover the computer screen with a towel or shirt.
15. Invert the first pond sample several times to mix.
16. Using the funnel pour 25-30mL of sample into the test tube.
17. Ensure the fiber optic light source is pressed up against the test tube.
18. Press the left mouse key/SAT-Pulse icon to take the first reading.
19. Remove the test tube from the stand and vortex briefly to mix.
20. Replace the tube in the stand and press the left mouse key/SAT-Pulse icon to take another reading.
21. Repeat steps 19 and 20 once more to collect a total of 3 readings.
22. Dump the sample into the waste container.
23. Repeat steps 15-21 for all remaining samples. Keep samples in the order that they were read.
24. After all samples have been processed save data analyze using the PAM data analysis template.

< Wipe down the outside of the test tube with a paper towel after every 6 samples are read to ensure the tube is clean.>

### 3. Required documents

#### 3.1. Input documents

<Input document and storage instructions>

<Input document number>

#### 3.2. Output documents

<Output document and storage instructions>

<Output document number>

### 4. Document control

#### 4.1. Revision history

R0 – Initial Release – <Nicole Heaps>	<Date>
R1 – <Cheng Fang>	<05/2013>

#### 4.2. Document approval

<Name>

<Approval date>

#### 4.3. Document reviewers

Cheng Fang  
Kari Mikkelsen

<05/2013>

### 5. Risk analysis

<Risk name>

<Mitigation plan>

<Owner>

<RPN>