Operating the Aquafluor Fluorometer

The *Aqua*fluor™ is a lightweight, handheld fluorometer/turbidimeter. Its dual channel capability allows the user to measure either *in vivo* chlorophyll *a* fluorescence or turbidity in one sample. However, only fluorescence measurements will be considered here.

General Information, Precautions and Cleaning

· The sample compartment can only accept 10mm square polycarbonate plastic cuvettes. Do not force oversized cuvettes into the sample compartment. This can damage the sample compartment.

· Use caution around solvents because they may attack the plastic case of the *Aqua*fluor.

· If a sample is accidentally spilled inside the Sample Compartment, you can invert the *Aqua*fluor to drain out the excess liquid. Then wipe the inside area dry with a clean soft towel or tissue.

· If extra cleaning is needed, use a mild detergent to dampen the towel for cleaning. Do not submerge the *Aqua*fluor in water.

· Do not expose the *Aqua*fluor to temperatures outside the specified range of 5 to 40 C, or damage may occur to the unit.

Instrument Power Up

To turn on the *Aqua*fluor, press the <ON/OFF> button. After a 5 second warm up, the *Aqua*fluor is ready for operation. Pressing the <ON/OFF> button again will turn the unit off or if left idle for 3 minutes the unit will turn itself off to save battery power.

The *Aqua*fluor has 2 detection channels that are configured as two one fluorescent (Chlorophyll) and one turbidity channel. The appropriate channel is selected by pressing the <A/B> button to toggle between the 2 channels. The display will show a label in the lower left corner of the Home screen to identify which channel is activated.

Calibration

A secondary standard is one that contains a different type of fluorescent material than your samples. For *in vivo* chlorophyll readings, a Secondary Standard (PN 8000-950) is used for calibration. When a Secondary Standard is used for calibration, the *Aqua*fluor will give relative sample readings that are proportional to the measured fluorescence. In some cases these relative sample readings can be correlated back to actual concentrations that are

determined later. For example, this is commonly done for *in vivo* chlorophyll monitoring applications; however, this will not be done for PLANS measurements.

Calibration procedure

It is recommended for best accuracy, that you always calibrate before performing your sample analysis. The *Aqua*fluor will save the calibration settings for each channel until a new calibration is performed.

If the temperature of your samples or the *Aqua*fluor changes significantly, the readings may show a small shift and in this case, you should consider recalibrating. The solid secondary standard is useful for checking the reading stability over time and can also be used to recalibrate if needed.

Assign a Calibration Standard Value. This defines the numeric value that you want the standard to read. For calibrating with the cholorpyll secondary standard set the value to 10. This is a relative value and its units are arbitrary Relative Fluorescent Units (RFU).

1. Press the <STD VAL> button.
2. Use the up and down arrow buttons to set the standard value. Holding down either arrow button down will allow you to change the value using fast scrolling.
3. When finished, Press the <ENT> or <ESC> button to accept the value and to return to the Home screen.

Perform the Calibration

1. Press the <CAL> button.
2. Press <ENT> to start the calibration.
3. Insert your blank sample and press <ENT>. The *Aqua*fluor will average the reading for 10 seconds and set the blanking zero point.
4. Insert the standard sample and press <ENT>. The reading is averaged for 10 seconds and the Standard Calibration value is set.
5. Press <ENT> when the calibration is complete to accept the calibration. If <ENT> is not pressed within 10 seconds, you will be asked if you want to abort the calibration. Press the up or down arrow button to abort or accept the calibration, respectively.

If at anytime during steps 1-4 you want to stop the calibration, press <ESC>. This will return you to the Home screen and will default the instrument to the previous calibration.

Sample Analysis

1. Insert your sample. The orientation and cleanliness of the cuvettes can have an impact on the accuracy of your results. (See below)
2. Press either <READ> button. The instrument will measure and average the fluorescence signal for 5 seconds.
3. The reading result will be displayed on the top line of the Home screen.
4. The top left corner will then display “WAIT” for 5 seconds. Once “WAIT” disappears, another sample reading can be performed.

Handling Samples

1. Take care not to spill samples into the sample chamber. Wipe up any spills promptly.
2. The cuvette MUST BE DRY on the outside when taking readings. Any Moisture or condensation on the outside of the cuvette can affect the reading.
3. Fill the cuvette with at least 2mL solution volume or at least 50% full. Significant error in the readings can result if the cuvette contains less than this minimum volume.
4. The *Aqua*fluor is very sensitive and even small amounts of material from a previous sample may contaminate the sample and result in errors. Use a clean cuvette for all readings. If you are using the same cuvette for your samples it is very important that you thoroughly clean the cuvette between samples. A good way to confirm the cuvette cleanliness is to read a blank solution. If the reading is higher than the normal blank reading, the cuvette is not clean.
5. Any bubbles in the sample will affect the readings. Take care not to introduce bubbles into samples. Remove any bubbles by lightly tapping with your finger on the outside cuvette wall or cover the top of the cuvette and tilt the sample to help dissipate bubbles.
6. The orientation of the cuvette in the sample compartment can give slightly different readings especially for low concentration samples. This is due to variations in the walls of the cuvette that are not readily visible to the eye. We recommend that the cuvette be marked at the top on one side and positioned in the sample compartment the same way each time for best results.