Phycocyanin Calibration Standards

Background

Much like chlorophyll, phycocyanin harvests light energy for photosynthesis, but unlike chlorophyll, which occurs in many organisms from microbes to plants, phycocyanin mostly is found in cyanobacteria (blue-green algae). Phycocyanin is the blue fluorescent protein (excitation 600-620 nm, emission 640-650 nm) that gives these organisms their distinctive color. Therefore, phycocyanin fluorescent measurements are used in environmental sampling to estimate the abundance of cyanobacteria. Though relationships between phycocyanin measurements and cyanobacterial concentrations have been determined, with 1 ppb of phycocyanin roughly relating to 1,000 cells per milliliter, caution must be used with this estimate because changes in sample turbidity, temperature, light intensity, and cyanobacterial species have profound impacts on measurements (Brient et al. 2007; Bastien et al. 2010).

Product Description

Phycocyanin has been enriched, but not purified, from toxic cyanobacterial extracts. Appropriate protective equipment should be worn to protect from accidental cyanotoxin exposure. The concentration of phycocyanin in the extract has been determined using a fluorometer calibrated with pure phycocyanin using an equation from Bennett and Bogorad (1973). The extracts were prepared in a solution containing stabilizers then freeze-dried and sealed under vacuum. This process results in the final appearance as a pale-blue or white powder. Note that the concentration of phycocyanin in this powder is too low for the powder to be intensely colored.

Handling

Upon receipt, the vials containing the freeze-dried phycocyanin powder should be stored in a normal freezer (-20°C) and protected from light. Once dissolved, it should be used immediately because the phycocyanin slowly loses fluorescence. For cuvette style fluorometers, the low-volume standard will have been provided, and can be dissolved by adding 6 ml of water to the vial. The final concentration will be shown on the Certificate of Analysis that accompanies the product. If measuring equipment is not available, add water up to the neck of the vial (see picture) to obtain 6 ml. The low-volume phycocyanin calibrator already contains a stabilizer in the vial.

If the high-volume product has been purchased for calibration of a submersible probe, 25 g of stabilizer has been shipped with the product. This stabilizer should be dissolved to a final volume of 500 ml with water, and a portion of the stabilizing solution should be used to dissolve the phycocyanin that is in the vial so that it can be transferred to the final volume of 500 ml. *Do not dissolve the large-volume calibrator without the stabilizer, and do not dilute the stabilizer below 5%.* The concentration shown on the Certificate of Analysis represents the concentration in this 500 ml volume.

Should the user desire more concentrated calibration solutions, the volume of water can be reduced and the final concentration calculated accordingly. The higher concentration of stabilizer in these situations will not interfere with fluorescence. However, if the user desires a *more dilute* solution, a second 25 g portion of stabilizer can be provided.
free of charge. This extra stabilizer can be brought to 500 ml final volume with water and used to make dilutions. It is paramount that dilutions not be made with plain water that does not contain the stabilizer; the calibrator will lose fluorescence rapidly when dissolved without the stabilizer.

