NAME: Perkin Elmer Lambda 35 UV/Vis Spectrophotometer
S/N 101N7060404

1) Introduction
   The Lambda 35 is a double beam UV/Vis spectrophotometer from Perkin Elmer, packing pre-aligned Tungsten and Deuterium Lamps. It has a wavelength range of 190-1100nm and a variable bandwidth range of 0.5 to 4nm.

2) Calibration/Maintenance
   Internal wavelength accuracy and baseline stability tests are performed by the user on a regular basis (at least once per week). A Holmium Oxide standard test is also performed on a regular basis (at least once per week) to check wavelength accuracy. Currently, a service contract with Perkin Elmer exists to perform preventative maintenance or other services.

3) Measurement
   The method described based on Roesler (1998). An ESCO metallic coated Neutral Density filter (D=1.0; S71000) is kept in the reference beam in place of a GF/F filter to provide balance between the sample and reference beams, which allows for the transmission of 10% of the reference beam (comparable to transmission allowed by a GF/F filter). The neutral density filter is made from BK7 optical glass, which allows for controlled attenuation in the UV. Blank filters are soaked in 0.2µm-filtered ASW for at least 30 minutes. Scans are performed between 300-800nm with a 2nm Slit Band Width (SBW), 1nm data interval and 240nm per minute scan speed.
   a. The system is baselined using air. An air scan is performed to assess the stability of the system. Scan should measure 0.000 absorbance units ±0.005. If not, system should be baselined again.
   b. The neutral density filter is placed on the source side of the reference beam window using black electrical tape. The system is baselined again, with air in the sample beam.
   c. A moistened blank GF/F filter is placed on the detector side of the sample beam window and the system is baselined again.
   d. Immediately following the baseline, without removing the blank filter, a sample scan is performed. The scan should be flat. If the scan is not flat, then the system is baselined again. This particular system is noisy in the UV (300-350nm). The filter is rotated 90 degrees and scanned again.
   e. Moistened blank filters (3-4) are scanned periodically throughout the day to monitor instrument drift.
   f. For samples, three to four drops of artificial seawater are placed in a petri dish. The sample filter is placed biomass up onto the water droplet. The sample filter is allowed to thaw for 5 minutes before measurement. The petri dish is covered with the lid and foil to protect from the light.
   g. The sample filter is placed on detector side of sample beam and is measured three times at 0, 90 and 180 degrees.

h. The diameter of the biomass is measured using calipers (Fisher Scientific Digital Caliper, model # 14-648-17)
i. The extraction protocol is based on Kishino et al. (1985). Briefly, the sample filter is placed in glass filter cup and stem. Approximately 10-20ml of 95% methanol/5% ultrapure water is gently added to the filter cup and immediately filtered at 5-7 psi. After the first 10-20 ml are filter through, the valve is closed and another 20 ml are added to the filter cup. The sample is allowed to soak for 20 minutes then filtered again. Another 20 ml methanol are added to the cup and the sample is allowed to soak for at least another 20 minutes. Filter cups are covered to prevent debris from contaminating the sample.
j. After extraction, the last 20 ml of methanol are filtered through, and the filter is rinsed with 20 ml of ASW. The filter is not allowed to dry.
k. The moistened, extracted filter is scanned again using the protocol described above.

4) Data processing
   a. Mean of three A_p and A_d scans is calculated
   b. Blank scan closest to the sample scan is subtracted across spectra from the mean A_p and A_d scans (if there is instrument drift).
   c. The blank-corrected A_p and A_d scans are null corrected by subtracting the absorbance value at 750nm from the absorbance at all wavelengths (apcorr)
   d. Absorption coefficient is calculated using the following equation apcorr*[2.303*100/β*pathlength]

      Pathlength = volume filtered (cm^3)/area of filter (cm^2)
      Area of filter = $3.14*((Diameter/10)/2))^2 = \pi r^2$
      Diameter was divided by **10 to convert mm to cm and by 2 to get radius**
      $\beta = 2$ (Roesler, 1998)

      $A_{ph}=A_p - A_d$

5) Data reporting
   Each SeaBASS submission of Ap scans will include the following:
   a. Blank-corrected raw absorbance of both ap and ad
   b. Standard deviation of rotation scans for both ap and ad
   c. Absorption coefficient calculations for each replicate (where applicable) for ap, ad and aph
   d. Standard deviation of absorbance of all blank filters measured throughout the analysis period

   Note: files that contain both replicates and more than one column of blank error indicates that replicates were analyzed on different days.

6) Reporting Notation
   abs_ap = raw total absorbance with blank subtracted (no null correction)
   stdev_abs_ap = standard deviation of 3 filter rotations
   abs_ad = raw Ad absorbance with blank subtracted (no null correction)

stdev_abs_ad = standard deviation of 3 filter rotations
ap = absorption coefficient (null correction included)
ad = absorption coefficient (null correction included)
aph = absorption coefficient ($A_{ph}=A_p - A_d$)

Kishino, M.N., Takahashi, N., Okami, N., and S. Ichimura, 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bulletin of Marine Science. 37, 634-642.

Roesler, C.S. (1998): Theoretical and experimental approaches to improve the accuracy of particulate absorption coefficients derived from the quantitative filter technique. Limnology and Oceanography 43: 1,649-1660.