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/week). A Holmium Oxide standard test is also performed on a regular basis to check wavelength accuracy. Currently, a service contract with Perkin Elmer exists to perform preventative maintenance or other services.

3) Measurement
   The method described follows that of the NASA Ocean Optics protocols (Volume IV, Rev. 4). Blank GF/F filters moistened with artificial seawater (ASW) are placed on the detector side of both the sample and reference beams. The blank filter is scanned three times at 0, 90 and 120 degrees. During sample scans, a moistened blank filter is left in the path of the reference beam. This reference filter is remoistened after each set of sample scans. Blank filters should be soaked in 0.2µm-filtered ASW for at least 30 minutes before use. Scans are performed between 300-800nm with a 2nm Slit Band Width (SBW), 1nm data interval and either 120 nm or 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. Moistened blank GF/F filters are placed on the detector side of the sample beam and reference beam windows.
   c. The blank GF/F in the sample beam is scanned three times at 0, 90 and 180 degrees. The reference filter is kept in the reference beam.
   d. 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. Petri dish is covered with the lid and covered in foil.
   e. The sample is placed on detector side of sample beam and is measured three times at 0, 90 and 180 degrees.
   f. The diameter of the biomass is measured using calipers (Fisher Scientific Digital Caliper, model # 14-648-17)
   g. For extraction, the sample filter is placed in a glass filter cup and stem. The filter is initially rinsed with 100% methanol warmed with hot tap water and immediately filtered at 5-7 psi. Approximately 20 ml of 100% methanol ultrapure water was gently added to the filter cup and allowed to soak for approximately one hour. Filter cups are covered to prevent debris from

contaminating the sample. After extraction, the methanol is filtered through, and the filter is rinsed with 20 ml of ASW. The filter is not allowed to dry.

h. The moistened, extracted filter is scanned again using the protocol described above.

4) Data processing
   a. Mean of three A_p and A_d (NAP) scans is calculated
   b. Blank scan closest to the sample scan is subtracted across spectra from the mean A_p and A_d scans
   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**
      β = 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
   stdev_abs_ad = standard deviation of 3 filter rotations (no null correction)
   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 and S. Ichimura, 1985: Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bulletin of Marine Science 37: 634-642.

Mitchell, B.G., M. Kahru, J. Wieland, and M. Stramska, 2003: Determination of spectral absorption coefficients of particles, dissolved material and phytoplankton for discrete water samples, In: Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Rev. 4, Vol. IV, J.L. Mueller, G.S. Fargion and C.R. McClain, Eds.

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.