TD-700
Laboratory Fluorometer

Application Note:
A Suggested Method for Using the TD-700 Laboratory Fluorometer with EPA Method 445.0

REQUIRED MATERIALS

- TD-700 Laboratory Fluorometer with Red PMT and 13mm x 100mm Round Test Tube Adaptor (P/N 7000-000).
- Daylight White Lamp (P/N 10-045).
- Chlorophyll Optical Kit (P/N 7000-961). Kit includes excitation filter (P/N 10-050R) and emission filter (P/N 10-051R).
- 13mm x 100mm Round Borsilicate Glass Test Tubes (P/N 10-031).

OPTIONAL MATERIALS

- Liquid Primary Chlorophyll *a* Standard (P/N 10-850).
- Solid Secondary Standard (P/N 7000-994).

PROCEDURE

1. Configure your instrument with a 10-045 Daylight White lamp, excitation filter 10-050R and emission filter 10-051R.
2. Turn on fluorometer and allow it to warm up for 10 minutes. Make sure the lamp is functioning by checking the lamp view port on the back panel of the instrument.
3. Make sure filters and filter cylinder are in the correct positions. For example, if you wish to use position “A”, the excitation filter should be placed in the hole marked “A EX.” The emission filter should be in the hole marked “A EM.” Then, place the filter cylinder in the sample compartment, with the “A” on your left hand side, as you face the front of the instrument.
4. Install the test tube holder in the filter cylinder. Make sure the silver peg is seated in the notch on the filter cylinder.
5. From the [HOME] screen, press [ENT].
6. For Mode, press [1] and select Multi-Optional by pressing the toggle key [⇔]. Then press [ESC].
7. For Cal Procedure, press [2] and select Direct Conc. by pressing the toggle key [⇔]. Then press [ESC].
8. For Units, press [3] and select desired units by pressing the toggle key [⇔]. Then press [ESC] twice.
9. Select Calibration by pressing [2]. Press [9], enter maximum range of 250 (chlorophyll-*a* linearity is maintained up to 250 ppb; samples above this concentration should be diluted for best accuracy) and then press [1].
10. Calibrate instrument with a known, pure chlorophyll-a standard (i.e. 100 ppb). Select [1] for number of standards or [2] through [5] if using more than one standard. Press [9] and enter concentration of standard when prompted. Then press [1]. Insert standard and press [*] to calibrate the instrument.
11. Blank with a 90% acetone solution. Insert into instrument when prompted. Corrected value for your standard is the R_b value.
12. Acidify standard by adding 0.15 mL of 0.1 N HCl for every 5.0 mL of standard and measure fluorescence in fluorometer. This is your R_a value.
13. Calculate $r = R_b/R_a$
14. Extract all filtered samples as described in EPA Method 445.0, rev. 1.2 (September 1997), section 11.1.
15. Measure fluorescence of all samples. The results obtained are your R_d values. These values are your chlorophyll *a* results without pheophytin correction. To correct for pheophytin *a*, proceed to step 16.
16. Acidify samples by adding 0.15 mL of 0.1 N HCl for every 5.0 mL of sample.
17. Measure fluorescence of acidified samples. The results obtained are your R_c values.
18. Calculate the corrected chlorophyll *a* and pheophytin *a* concentrations as follows:

    Chlorophyll *a*, µg/L = $(r/r-1)(R_d-R_c)$

    Pheophytin *a*, µg/L = $(r/r-1)(rR_c-R_d)$

    r = the before:after ratio of a pure chlorophyll *a* solution

    R_d = concentration of sample extract before acidification.

    R_c = concentration of sample extract after acidification
19. The concentrations of chlorophyll *a* and pheophytin *a* in the natural water sample are calculated by multiplying the results obtained in step 18 by the extraction volume (in milliliters) and dividing by the volume (in milliliters) of natural water sample that was filtered. Any other dilution or concentration factor should also be incorporated accordingly.