

## SLM AMINCO 8100 Spectrofluorometer Protocol

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Clark Lab

Location: Delia's office. Across from Nowak Office.

Program: Left Computer, OS/2, SLM 8100

- ❖ Pertactin: Molar abs. coefficient = (# trp) (5,500) + (# tyr) (1490) + (# cys) (125)

Trp = 9

Tyr = 7

Cys = 0

Molar abs. coefficient =  $59,930 \text{ M}^{-1} \text{ cm}^{-1}$

$C = A / (E L)$

C = conc. of protein (M) A = abs at 280nm E = molar abs. coeff.

L = path length (cm)

$C = A / 59,930$

MW = 61,400 g / mol

$C = 1.02 A = \text{mg} / \text{ml}$

Used 40 ug / ml of Pertactin for samples (this corresponded to 50 ul of an ion exchange fraction from Prep #1).

- ❖ Volume: 900 – 1000 ul in cuvette (semi-micro).

- ❖ Procedure:

1. Sign in to Fluorometer Log-In sheet located next to fluorometer. Follow instruction on sheet.
2. Lamp needs to be warmed up for 20-30 min prior to use.
3. Make sure everything is off including computer and monitor.
4. Flip switch to turn lamp on. Then push ignite button. Note that the light turns on and the fan is running. Allow time for warm-up.
5. Set slit widths to appropriate values.
6. Pull open red knob door located on the end of the monochromator near the lamp.
7. Turn on computer and monitor.
8. While booting up....
9. Turn on Photomultiplier tube cooling water.
10. Turn on water alarm.

11. Turn on cuvette bath.
12. Turn on instrument.
13. Back at computer press any key until the option menu appears. Choose A for OS/2. Then type y and press enter.
14. Close all windows that open at start.
15. Double click SLM 8100 icon in top left corner of screen. Program loads and instrument initializes.
16. Click user tab, login, choose user id.
17. File Tab : ASCII export setup. Click ASCII extension is txt.
18. Status: Leave this window open while using instrument.
19. Channels : EmL is already loaded, add EmL / Ref, and add Ref. OK.
20. Monos : Excitation 280, EmL 330.
21. Shutters : Excitation=Open, Check=Open Shutter for Acquisition, Emission Left=Open, Emission Right=Closed, Reference=Open.
22. Sensitivity : Ref click AR at Gain of 1 this will automatically set the reference. Put in a blank sample and click AR this will automatically set the EmL. May need to change the Gain from 1 to 10 or 100. Record Gains and HV for each.
23. Go to Applications tab : Emission scan : set lower and upper limits 300 and 400. Set integration, scan rate, # of repetitions. OK.
24. Go to Applications tab : Start Application : Select File name and type.
25. When finished go to File : Open. Choose search and change from \*.dat to \*.txt. Select files and click Copy. Type a: to copy to diskette.
26. Make sure all data is saved.
27. Exit SLM 8100 program and double click Dual Boot. Type y and enter. Computer will begin shutdown process. Meanwhile...
28. Follow fluorometer sheet for shutting things down in reverse order.
29. Then turn off computer and very last turn off the lamp and record the total time used.