

Perkin Elmer Lambda 40Spec Protocol

(written for determining chla concentration, but applicable for general use)

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1. Turn on the spec by pressing the green button on the back right corner of the machine. It requires at least 30 minutes to warm up before running your samples.
2. While the spec is warming up, you can open and edit your software method, and prepare your samples.

Creating your method

1. Log into the computer hooked up to the spec. Go to the start menu, program files, PE Applications and select **Lambda 40P**.
2. When the program opens the method window will appear. Select a known appropriate method or see details below for correct settings.
3. The window for that method will pop up and the scan tab will be highlighted in the lower margin.

Settings to check on the scan tab

- Start wavelength = 800 nm.
 - End wavelength = 300 nm
 - Number of cycles = 1
 - Data interval = 1.0 nm
 - Ordinate Max = 2
 - Ordinate Min = 0
 - Display = Serial
4. Now click on the **instrument tab** on the lower margin of the method window.

Settings to check on the instrument tab

1. Ordinate mode = A
 2. Lamp UV = ON
 3. Lamp Vis = ON
 4. Slit = 1.00 nm
 5. Scan speed = 240nm/min
 6. Smooth = 0nm
5. Now click on the **sample tab** on the lower margin of the method window.
 6. At the top of the window, change the **result file name** to something that you will recognize, such as the sample and julian date (ex: Chla6045).
 7. Set **Calculation Factor** to **factor**.
 8. On the upper toolbar, go to **utilities** → **configuration**. The configuration menu will pop up. Change the **data directory** to save file to appropriate location. Then click **OK**.
 9. Set **number of samples** to the number that will be run, typically 7.
 10. In the lower portion of the window there will be lines for the **seven samples** to be run, including their **Sample identity, factor** and **Sample info**. A typical calibration table would look like this:

Number	Identity	Factor	Sample Info
1	acetone	1.00	Acetone blank
2	Chla 1	1.00	Chla standard # 1
3	Chla 2	1.00	Chla standard # 2
4	Chla 3	1.00	Chla standard # 3
5	Chla 4	1.00	Chla standard # 4
6	Chla 5	1.00	Chla standard # 5
7	acid	1.00	Chla standard + HCL

Preparing your samples

1. Stuff you will need:
 - Fresh bottle of 90% acetone
 - Several disposable glass pipettes with bulbs
 - Small glass beaker and funnel
 - Aluminum Foil
 - 10% HCL bottle with pipette
 - Acetone waste beaker
 - Two 1cm quartz cuvetts found above the Spec.
 - A 100ml glass volumetric flask with stopper (found in the Fluorometer Calibration glassware box)
2. Open the spec and check if the cuvet holders inside fit your cuvetts. If they don't you will have to change them out. Find the holders that fit your cuvetts. Simply unscrew the ones inside and replace them with the proper holders.
3. From the -70°C freezer in lab 5 (lights off), get a 1mg Chla standard vial (*Anacystis nidulans*, NOT spinach) from a lot labeled with the current year. Record the lot number on the fluorometer calibration sheet. Note: The standard should always be kept in the dark to minimize degradation so it's best to have everything ready to go before you get it out of the freezer.
4. Fill your beaker with 90% acetone
5. (with the lights out) Gently snap the top off of the standard vial. Invert vial into the 100ml volumetric flask and tap gently to get the Chla crystals into the flask.
6. Using one of your glass pipettes, fill the standard vial halfway up with 90% acetone, taking care to rinse down the sides of the vial in the process but try not to contaminate the pipette with sample.
7. Using a separate pipette, remove the acetone in the standard vial and put it into the volumetric flask. Repeat the last two steps approximately 5 times or until you are satisfied that all of the standard has been transferred to the Volumetric flask. Do the same process with the snap-off top from the standard vial.
8. Once all of the standard has been transferred to the volumetric flask, fill it the rest of the way up with 90% acetone until the bottom of the meniscus touches the line on the neck of the flask.
9. Stopper the flask and cover it in Aluminum foil to minimize light exposure.
Note: It's a good idea to keep the stopper in whenever you aren't using the standard because the acetone will evaporate off and change the standard concentration.

Reading your samples

1. Using a fresh pipette, rinse out your two cuvetts with 90% acetone a few times. Then fill both with fresh 90% acetone and wipe down the outside with a kimwipe being careful not to get any fingerprints on the glass. It's best to touch them only on the corners or top/bottom.
2. Place the cuvetts in the holders in the spec and close the lid.
3. Back to the computer; Click the **autozero button** in the upper left corner of the screen.
4. It will make some cool sounds and then it will say **Please insert next sample: 'Blank'**. Just click okay. This takes a few minutes so be patient.
5. When it is finished, the **autozero button** will turn grey and the **green start button** will illuminate. Press **Start**.
6. It will say **insert next sample: 'acetone'**. Since you already have an acetone blank in there, just go ahead and click **OK**. The acetone spec should be a flat line until it gets to approx. 310 nm (acetone gives odd peaks in UV light).

Note: if you did not change the data directory in step 7 of "creating your method" it will not be able to save the scan and it gets unhappy. So, if you get an error message here you may need to do that step again and start again from Autozero.
7. When it says **insert next sample: 'Chla 1'** remove the front cuvet from the spec and waste the contents. Then fill it with your standard solution using a clean pipet. Wipe it down with a kimwipe (always) and place it back in the spec. Make sure that the cuvettes are oriented in the same direction every time a reading is made. Click **OK**. You should see a peak at about 662nm and a second at 430nm.

Note: To save on standard solution, each cuvet full of standard will be run twice before being replaced. For example: This first one will be used for both **Chla 1** and **Chla 2** scans. The cuvet will be emptied and refilled with standard solution for the **Chla 3** and **Chla 4** scans, then again for the **Chla 5** and **Acid** scans.
8. Then it says **insert next sample: Chla 2**. Simply click **OK**.
9. Then **insert next sample: Chla 3**. Remove the front cuvet and waste the contents. Rinse it out a couple times with 90% acetone. Then refill it with your stock solution just as before and place it back into the spec. Click **OK**
10. Continue along as detailed in step 16 until it says **insert next sample: 'acid'**. Put two drops of 10% HCL into the cuvet containing the standard. Close the lid and click **OK**.
11. When the last scan has finished, you will need to go back and add all of the scans you did to the same graph. To do this, go to the toolbar and click **View** → **Add spectrum**. A list of your scans will pop up. Double click on one and it will appear in the graph you already have open. A list of the scans on the graph will be in the lower margin of the graph window. Continue doing this until all of your scans have been added to the graph.
12. Now, highlight one of your scans from the scan list at the bottom of the window by clicking it. Then go to the toolbar and select **data handling** → **List**. Then Click **OK**.
13. The spectrum for that scan will appear in numerical format. The right column is the wavelength and the left column is the absorbance. Highlight the entire thing and then right click, **copy it**. Or "copy to clipboard" from the "file" drop down on the menu bar

14. Now open Excel and **paste** this into a new workbook. Save it to the 016 drive in the Chl folder.
15. Repeat the last three steps, adding each scan to a new worksheet in the workbook you created until all of the scans have been added. Be sure to change the names on the worksheets to match the **sample identity** of the scans.
16. Now, go through each of the scans in the workbook and record the absorbance values from each, at wavelengths 664 nm and 750 nm.
17. Open your fluorometer calibration sheet that you prepared earlier. Enter the values that you recorded for 664 and 750 into those spreadsheets, top left under 1-5 for OD₆₆₄**b** and OD₇₅₀**b**. Enter the acidified values below the table under the appropriate sample number (typically 5) for OD₆₆₄**a** and OD₇₅₀**a**. It should now give you the standard concentrations for the dilutions you need to make for the calibration curve. Save it.
18. Turn off the spec and clean up. You're done!