Plate Readers

MD SPECTAmax (PSC 563)

Every time you use the plate reader, you MUST sign the logbook.
Turn off the plate reader when you are done so the lamp doesn't burn out.

Contact: Debby Walthall 404-413-5363; dwalthall@gsu.edu

1. Turn off plate reader.

MD SPECTRAmax Plate Reader

General Information

The plate reader calibrates when you turn it on. Once done calibrating, you can use it. When machine is calibrating, the drawer will come out when the machine is ready.
No warmup time is needed as long as RT is all you need. If you need a specific temp. – allow 10 minutes for the plate reader to get to temp.
Turn off at least once a week. If you don't turn it off periodically, you can get a cache problem and an error message. If you ever get and error message – just turn machine off and then back on and it should be OK.
Keep drawer closed – punch drawer button on machine or use button on computer.
You can use plate reader as stand alone (no computer), but it's easier to use the computer. Use stand alone for cuvettes.
.pda – data file; .ppr – protocol file
100 – 300 µl in each well works best.
Avoid "V" bottom microtiter plates. Round or flat are OK.

Power on plate reader. Switch in the back on the right.
SoftMax Pro 4.7.1 (icon on desktop).

Overview of menus and buttons. For more details of each item – see the manual.

Menus and Command

1. File. Usual items – open, print and save experiment data files.
2. Edit. Usual items – copy, paste, delete and duplicate items. This also contains Preferences which has printer options and also communication (COM1)and saving files options.
3. View. Usual – choose what is shown and how it is shown.
4. Experiment. This allows you to create new sections within an experiment in addition to the default sections (note, plate, cuvette set and graph; not standard) and also to open a new experiment.
5. Control. Allows you to setup and activate instrument functions. Pretty much the same things shown on the Status Bar.
6. Assays. Allows you to choose the folder containing the protocols you have saved and shows these protocols below the divider. This also has a number of canned assays that you can open.
7. Various. The last menu item changes depending upon what section is active – either plate, notes, group or graph. Within each of these are various choices for example changing the X axis or Y axis on a graph or different ways of handling the plate.

Status Bar (buttons) – from left to right under menu bar

1. Instrument icon. Indicates that the instrument is communicating with the computer. If icon is "X'd" out - make sure the plate reader is on and if still "X'd" out – double click on icon and the preference box will open – make sure it's set on COM 1.
2. ____°C. Current chamber temp. This is the temp. in the chamber. This may be different than the temp. on the machine – this temp. is the temp. in the cuvette holder. They should be about the same though once the machine is warmed up.
3. Read. Click to start reading. The reading is based on the settings chosen in Instrument settings dialog box.
4. Ref. reference button. References a cuvette based on information entered in the Instrument Settings dialog box. The reference reading may be taken before or after the sample reading.
5. Incubation button (looks like a thermometer). Opens the Incubator dialog box. This allows you to set and regulate the temp. of the microplate chamber. *The incubator setting is independent of the protocol being run.
6. Automix button (squiggly line, like a sideways sum symbol). Shakes the microplate. This is a manual shaking of the plate and is separate from the automatic shaking set up in Instrument Settings.
7. Drawer button (last button). Opens and closes the microplate drawer.

Experiments. SoftMax Pro collects data from 1 or more microplates and stores it in a single data file, using the same or different instrument settings.

Sections. Part of the SoftMax Pro experiment window intended to perform a specific set of functions. To do anything with a Section, it has to be active. Just click anywhere on the inactive section and this will activate it. More than 1 of each of the section types can be created. See Experiment under the Menu list above. To delete a section (active only), use the Edit/Delete command; just remember that if you have any data in that section it will be lost. To open and close a section, use the sideways triangle/arrow on the left side of the tool bar of each section. You can open a section as a separate window by double clicking the section icon on the left next to the arrow.

1. Notes. You can record information pertaining to the experiment.
2. Plate #. This sections shows a grid of cells which corresponds to the wells of the microplate. To the right are the current instrument settings. This is where you define a template to be used for analysis, determine instrument settings and choose how you want the data displayed.
3. Cuvette Set #. Determines how the cuvette will be read, defines the template to be used for analysis displays the data as it is acquired and defines how the data will be displayed.
4. Standards. Actual values including Std. Deviation and mean.
5. Unknown. Actual values including Std. Deviation and mean.
6. Unknown_Dilution. If used, will show actual values
7. Control. Actual values including Std. mean.
8. Standard curve. Graphs automatically when you have labeled the wells in the Plate section.

Data Collection

1. File/New. Opens with a Default Basic Endpoint protocol.
2. Click on the Plate section to make it active and open it. Following are buttons in order left to right.
    a. Setup button. Opens Instrument settings. You have 3 experimental choices Endpoint (a single reading at the end of an assay at a single or multiple wavelengths), Kinetic (data is collected over time with multiple readings taken at regular intervals) and Spectrum (values reported are optical density and can scan over a range of wavelengths). See below for specifics about Setup.
    b. Template button. Allows you to create or edit the template.
    c. Reduction button. Reduce raw data to a single number based on formulas. View with or without plate blank subtraction or cuvette assay blanking. You can modify wavelength combination settings as well as the data mode. This is where you can turn off Pathcheck after the read if you want to.
    d. Graph button. Enlarges selected wells to a well graph display – used only for Kinetic and

Spectrum modes.

e. Display button. Gives you display choices. Raw data, reduced data, grayscale, threshold, ranged or a combination (see below).
f. Zoom well button (looks like a graph). For kinetic and spectral scans only. You can enlarge a plot for 1 or more wells simultaneously in real time or when run is done.
g. Mask button. Removes the selected well from data analysis.
h. Printer button.

Endpoint. Options are on the panel to the left. As you select different options (click on title), the choices on the right change.

1. Wavelength. Remember, this machine is a no filter system, so you can read from 190 – 1000. There are 6 wavelengths in a drop down menu that you can select. If your wavelength isn't in that list, just type it in. You can also select the # of wavelengths to use. For example, if you change the # to 2 – 2 boxes appear and you can do a 260 and a 280 reading.
2. Pathcheck. If the volumes in your wells are different, you could get different OD's. Pathcheck senses volume and adjusts the OD to volume.
3. Automix and blanking. You can have the plate shake just before it's read – probably a good idea. You can also decide whether you want the plate to just shake before 1st read or between reads – depends on how many samples you have. You can also pre-read the blank plate. This corrects for well to well variability. MD never uses this setting, just have a blank in a couple wells. MD recommends using whatever buffer you use in your assay as the blank.
4. Autocalibrate. Default is on and should be left on. Turning this off will allow the read to go faster though.
5. Assay plate type. Leave at 96 well
6. Wells to Read. You can select only the wells with samples, which can speed up the read. Highlight the wells you want to read. Just remember that the wells not highlighted will NOT be read. MD recommends highlighting all the wells to avoid mistakes – it doesn't take much longer. Reader reads 8 wells at a time.
7. Speed read. Less precise. Don't really need this, the reading goes in 7 sec. Instead of 9 sec.

Kinetic. Same options as Endpoint except it has a run time and no pathcheck.

1. Timing. Set total time to run and the interval to take a reading in sec. After these 2 are set, the # of reads will be displayed.
2. Rest of selections same as above.

Spectrum. Same options as Endpoint except wavelength

1. Wavelength. Enter the wavelength you want to start with and the wavelength you want to stop and # of steps in run.
2. Rest of selections same as above.

Read. After parameters set up, put plate in plate reader, close drawer and press Read.

Data Analysis. The following is for Endpoint only. If you need Analysis information for Kinetic or Spectrum analysis, let me know.

1. After the plate is read, the information is displayed in the active Plate or Cuvette section in a grid array (microplate format). The data from Endpoint readings is raw OD numeric values. All the following choices affect only the manner in which the data is displayed – the *raw* data is not changed. This permits you to optimize the display after data is collected.
2. Highlight a set of wells. Select Template button or double click the highlighted area. A Template dialog box opens.
    a. Group. Select sample type in drop down menu (std., controls, unk).
    b. Edit. Change a previous sample type
    c. Delete. Deletes what you set up. (remember, the raw data is not deleted).
    d. Series. If you have replicates of different samples. Another dialog box opens. You need to select how the replicates are displayed (4 choices), # of replicates (click on # in box and arrows appear so that you can change the #), you can also set up any dilution series

you might have including concentrations for standards (needed to generate std. Curve). Select units (pull down menu); type in starting value; select "step by" – pull down menu with +, -, x or /; type in # to use for steps. Ex. To use 1 ug/ml, type in 1 and then 10.

e. Assign. You can label a well or a series of wells.

3. Data reduction changes the way in which data is displayed and also affects the values used in calculations. The raw values remain unchanged.
4. Display Dialog Box. You can select the display button. This allows you to change the way the data is presented. Available choices depend on which section is active and which read mode you chose (Endpoint, etc.).
    a. Data can be displayed normal (usual, rows labeled A – H and columns labeled 1 – 12, wells are small and data is shown with fewer digits), vertical, Rotated (plate is rotated 90^{o} clockwise), Large (larger size plate view), Interweaved (this skips every other well).
    b. Select raw data or reduced data. The reduced # is based on the settings defined in the Reduction dialog box. For example, you can do a blank subtraction or wavelength reduction. You can view the data with the raw data or with both raw + reduced or reduced only. Select "With reduced number".
    c. You can view the data by changing Options – Display. The choices are Threshold - shows data depending on the high or low limit you have set: a + (above hi limit) or a * (within limits or a – (below low limit); Ranged – also sets the high and low limits, but displays the data proportionally to integer values from 0 – 9 and also values above limit are + and values below limit are -; gray scale – displays data in 8 shades of gray, with colors changing from light for values less than or equal to the low limit to dark for values greater than or equal to the high limit.
    d. Graph options. Type of graph, ht. Of graph etc.

You can stack plates; you can do different things on the same plate. Experiment/New plate. Set up a new plate with different parameters. You can link the 2 plates.
You can also copy data from 1 plate, create a new plate and paste the data from the 1st plate into the new plate. Now you can do different things to the same set of data.
You can also use the standards from another plate.

Additional Sections:

5. Standard. Displays a table that includes: Sample name, Concentration, Back Calc Conc, Well #, value (OD), mean value, std. Dev. CV%.
6. Unknown. Displays a table that includes: sample name, Well #, value, result, mean/result, std. Dev. CV%.
7. Unk_Dilution. Sample name, Well #, value (OD), Result, mean result, std. Dev., CV%, Dilution, adj Result.
8. Control. Displays a table that includes: Sample, well, sample #, value (OD), mean value
9. Control.
10. Standard Curve. Automatically done when well labeled std. And values are entered. Concentration, Back Calc Conc., well #, value (OD), mean value, std. Dev., CV%. You can change fit to semi-log etc. if needed.

Mask. You can mask a data point if it messes up your standard curve. Either go to Plate/Mask or click on Mask button. Remember you haven't lost the data – it is just hidden. To remove Mask, just reselect button after highlighting well.
Y-Axis. 2nd button to right of Fit drop down ("l"). setting (minimum values, maximum Values on Y-axis, log/linear, show gridlines, show tick marks) and label graph.
X-Axis. 1st button to right of Fit drop down("-"). setting (minimum values, maximum Values on X-axis, log/linear, show gridlines, show tick marks) and label graph.

DNA

Use a cuvette or a UV transparent plate (96 well U plate; Costar, Corning – cat.#: 3635 (www.corning.com/lifesciences) when the OD you need to use is below 390.

Using UV transparent plate.

1. DNA limit of quantitation = 100 ng/well in a volume of 300 µl
   DNA limit of detection = 50 ng/well in a volume of 300 µl.
2. Use Pathcheck
3. Extinction coefficients for DNA analysis:

H_2O = 38.1 ug/ml
TE = 44.9 ug/ml
TE + saline = 50 ug/ml

4. Sample volume in a 96 well plate: 100 µl to 300 µl
5. Thoroughly mix sample and buffer in well. This is the #1 reason for variability of your results.
6. Determine plate background
   a. File/New
   b. In plate #1, click on the Setup button, choose, Endpoint, # of wavelengths = 2, set Lm1 to 260 nm, set Lm2 to 280 nm, strips set to Entire Plate, Choose 96 Well plate. Pathcheck Off. All other settings at Default.
   c. In plate #1, click on the Template button. With your mouse, highlight the entire plate grid in the template section, go to the Group pull down menu and select Blank. Click OK.
   d. Fill a clean microtiter plate w/ ~200 µl. of dH_2O in all the wells.
   e. Put the plate in the drawer of the reader. Click the READ button.
   f. At the end of the run, you will see a section in the bottom left-hand portion of the plate #1 that indicates "Plate Blank Used Lm1=,LM2=". These are you plate background constants that you can use for the entire lot of those plates. You will need to remeasure these values if you use a plate from a different lot or a different manufacturer. Write this # down on a slip of paper.
7. Assay Protocol. You can create your own protocol or use a pre-programmed protocol, make your modifications and re-save it as your own protocol. The most commonly used one is "DNA Background Constant".
   a. Assays/Nucleic Acids/DNA Background Constant
   b. The Notes section describes all the parameters of the protocol if you are interested.
   c. Go to Plate #1. Click on the Setup button. Most of the buttons are already chosen for you. Click on Pathcheck. This is whree you will type in the plate background constants that you have measured.
   d. Go to Automix, click on Automix before 1st read.
   e. Select 96 well plate
   f. Leave the rest at default.
8. Setting up the Sample Template with no dilutions.
   a. In Plate #1, click on the Template button.
   b. Highlight with your mouse the wells that contain your buffer only samples. From the Group pull-down menu, choose Blank. This will average the OD's from all of the wells that you have selected as Blank and subtract that average from all of the sample wells on the plate.
   c. Highlight the wells that are your Samples, go to the Group pull down menu, select Samples. You will need to map out which of your samples are individuals, duplicates, etc. You will need to highlight portions of the plate at a time in order to do this. See above for details about setting up replicates.
9. Setting up the Sample Group Table
   a. In the Group Table, below the Plate #1 called Samples, you will need to select your extinction coefficient. By default, it is set for H_2O. If this is not the value that you are using, double click on the name in the grid for "ExtCoell". Type in Summary #2 for TE, Summary #3 for TE + saline or simply type in whatever number you would like to use

instead of typing in Summary #X.

b. To save this as a permanent protocol, File/Save As, give it a name, under "Save as Type" select .ppr which will designate this as a protocol file. You can save this to any directory that you would like. If you save it in Program Files, Softmax PRO, it will be available as a choice in you Assays pull down menu.

10. Reading the plate.
    a. Prepare you DNA sample plate, put the microtiter plate in the drawer of the instrument, hit the READ button.
    b. The OD's in the Plate #1 section are in the order of Lm1 = 260nm, Lm2 = 280nm.
    c. All of your calculated results are in the Samples Group Table.
    d. Go to File/Save As, name it, save as a .pda file. This will save your data along with the protocol settings. You can modify the protocol file or the calculation fields but you cannot modify the raw data.
11. For information on setting up a Sample Template with Dilutions, e-mail Debby: biodaw@panther.gsu.edu

Using Cuvettes

1. Assay/Nucleic Acids/cuvette
2. You have to set REF first. Put H_2O or buffer into a cuvette and place in cuvette slot.
3. Select REF button. Now under each cuvette sample, under the Ref., you will see a date (changes from no reference to date).
4. Place sample into cuvette
5. Select READ
6. Once sample read, value (OD) automatically goes into A1
7. Next sample. READ. Value automatically goes into A2.
8. Data is found in cuvette Data section. Samle name, cuvette #, OD values 230, 260, 280; Ext. Coef, conc. Norm, con. dil., Conc., 230/280, 230/260, 26/280 norm., 260/280
9. To see the formula for each of these, select a column and the formula appears in the section header or double click on the column heading and the formula box opens. This is where you would make any changes to a formula – for ex. Changing the Ext. Coef.