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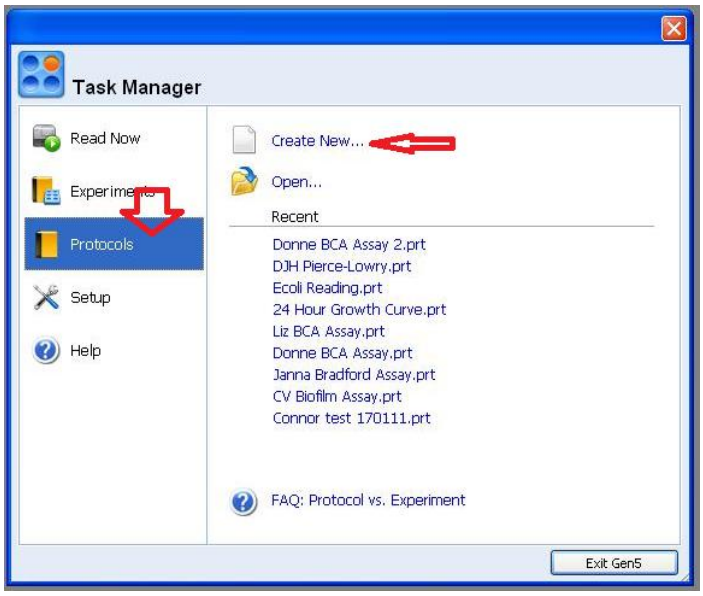
Plate Reader Quick Guide

BioTek Synergy HT

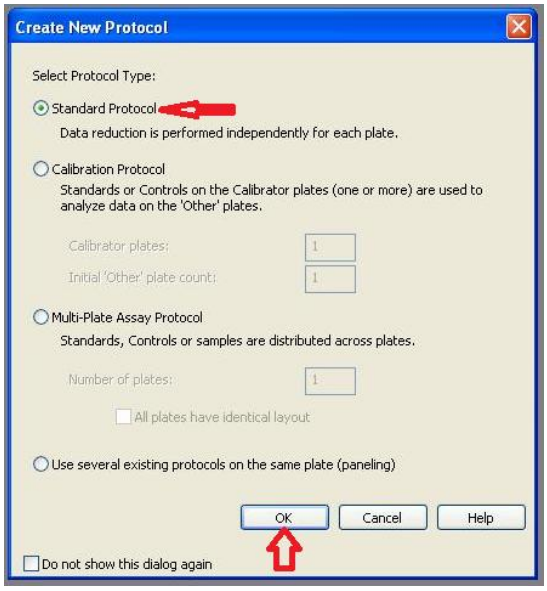
ver. 1.2

1. Turn on the plate reader and then open the **Gen5** software, which controls the plate reader, by double clicking on the icon in the upper left corner of the screen.

2. If you are making a new protocol, click on "Protocols" on the left of the menu and then click "Create New…" to the right. You can also select an older protocol here for modification.



3. Select "Standard Protocol" and click "OK". See the machine's manual if you are doing more advanced Assays.



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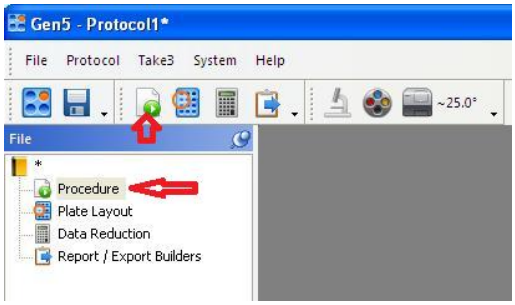
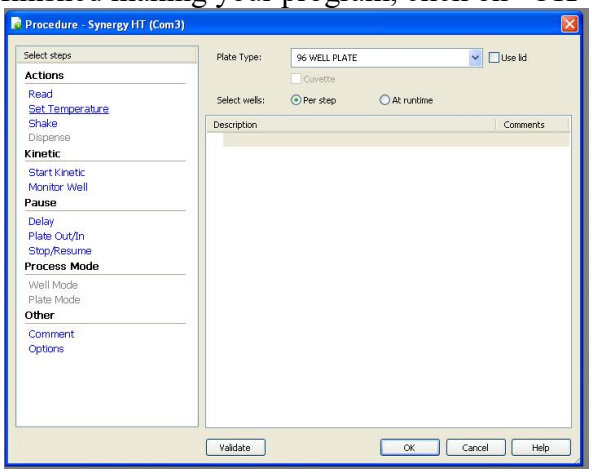
4. The Gen5 software will then be open with no more menus or boxes to guide you. To build your procedure you can click on the procedure button (white paper with a green arrow on it) or double click on the word “Procedure”.



4a. The Procedure box will let you create your own program, much like a thermocycler. The steps are to the left, and you can select them in any order that you prefer. When you have finished making your program, click on “OK”.



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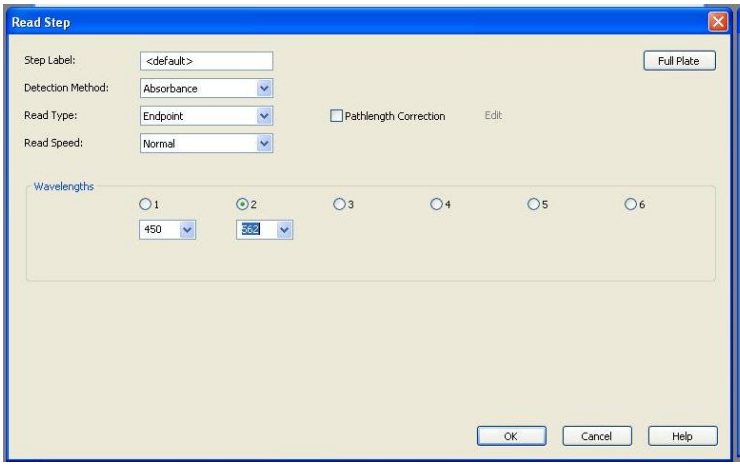
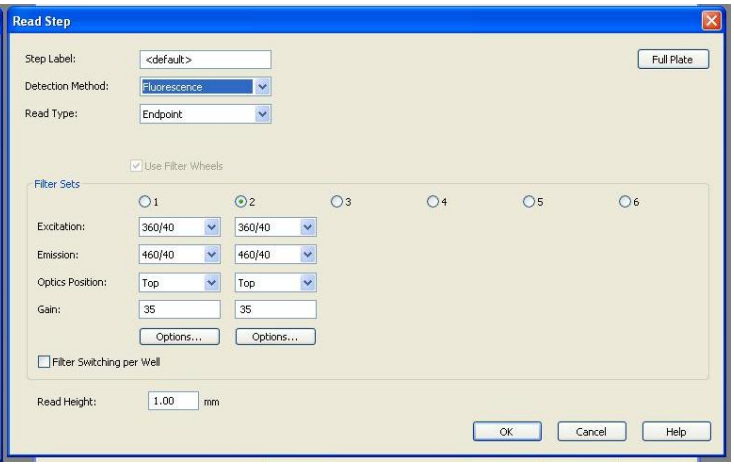
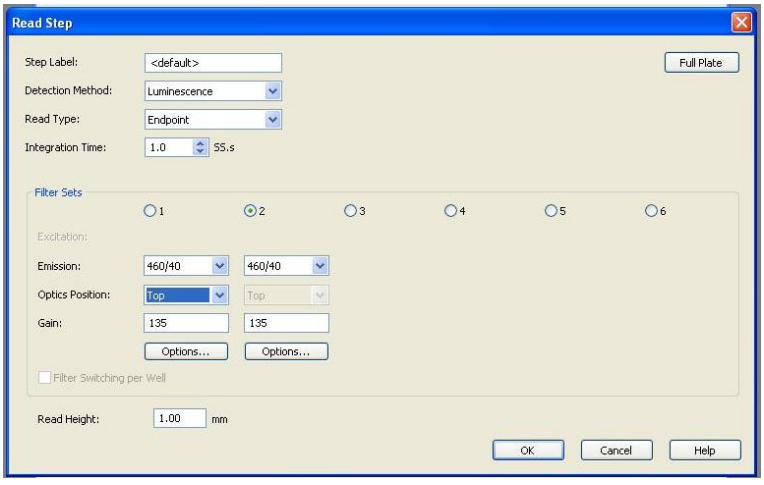
4b. The "Read" step under actions is, not surprisingly, the step where data will be collected. Clicking on read will open up a new box, which will allow you to choose the type of detection you are doing: absorbance, fluorescence, or luminescence. Multiple wavelengths (up to six in total) can be measured, and there will be appropriate selection pull-down menus depending on what you are doing (*e.g.*, there will be excitation and emission options if you are doing fluorescence). Wavelengths from 200-900 nm are available, although only some wavelengths will show up in the pull-down list. You can enter your desired wavelength by highlighting the entry box and then simply typing it in.

The Read Step options will look like this:







After finishing setting up your Read step, click "OK". You will go back to the main procedure page.

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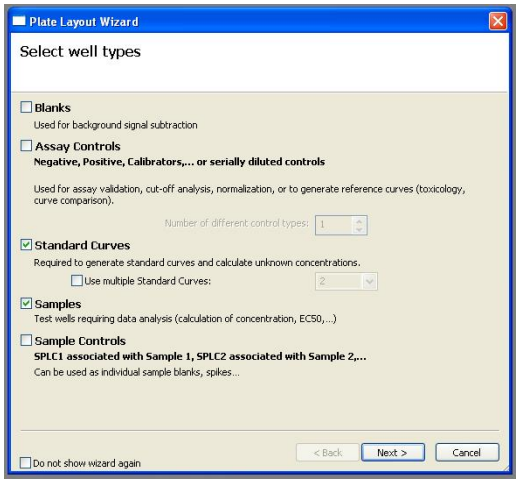
5. In order to set up the plate, click on the "Plate Layout" button (a plate with a little red circle) or double click on the "Plate Layout" in the left side of the screen.

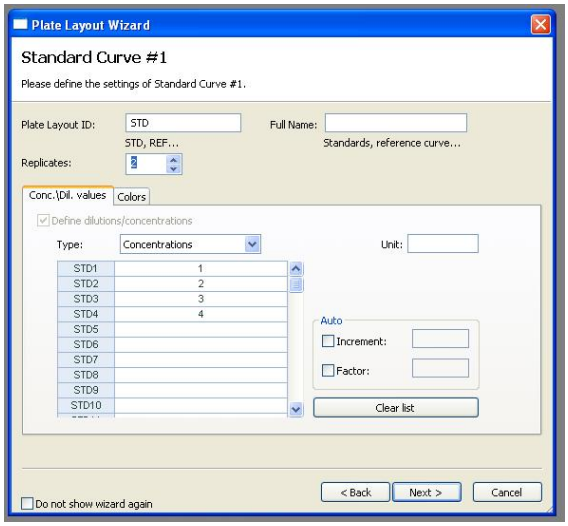
5a. The "Plate Layout Wizard" will now open and give you options for setting up your plate. You have several non-mutually-exclusive options here, which can be selected by clicking the checkbox in front of them.



Likely, you will choose "Standard Curves" and "Samples" for most of your experiments. Keep in mind that this is not a requirement, as you can always analyze your data on your own after the readings have been made. This is just to tell the software what each well is in case you want the software to analyze your results for you.

Click "Next >" when you are ready to proceed. The wizard will then guide you through setting up the standard curve values.

5b. Set up the standard curve by telling the wizard what each standard curve sample will be. You can name them (STD is default) and then select the number or replicates. Select the type (*e.g.*, Concentrations) and then you can write in the unit. Type in the values for each standard that you want to use; blank values will be ignored and not added to the plate. (*e.g.*, the example to the right would only have 2 replicates each of 4 standards).



Click "Next >" when you are ready to proceed.

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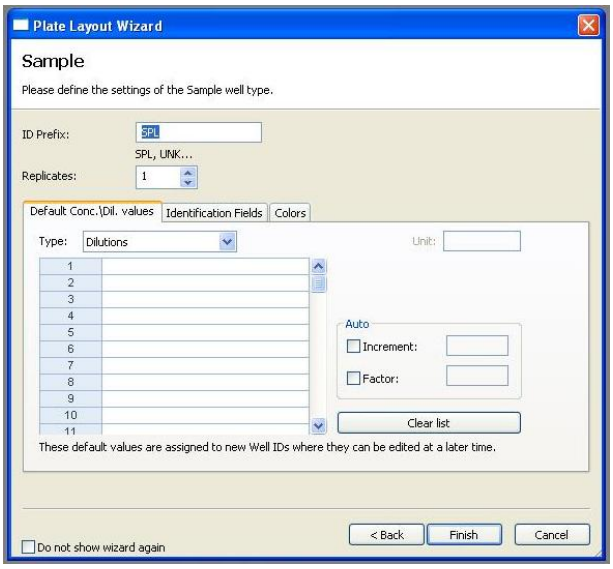
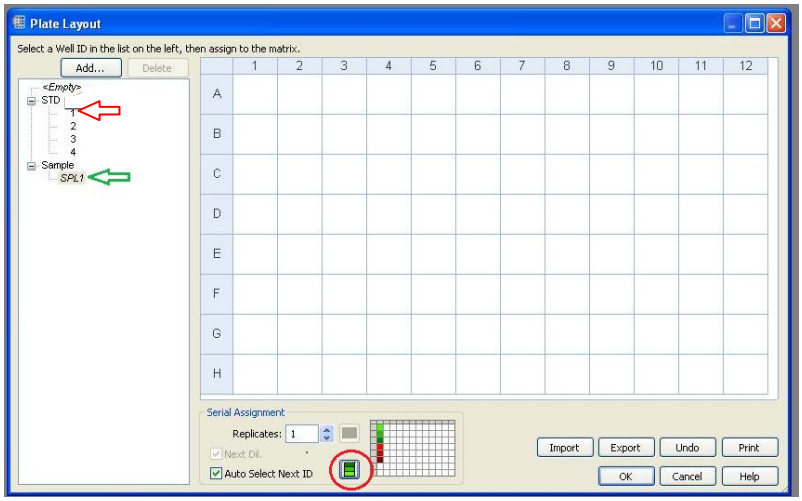
5c. Set up the samples by telling the wizard what each sample is. You can name the samples (SPL is default), and have multiple names by separating them with a comma. Enter the number of replicates, if you have more than 1 of each. You can add other information, such as dilution factors, for your various samples.

Click "Finish" when you have completed the Plate Layout Wizard.

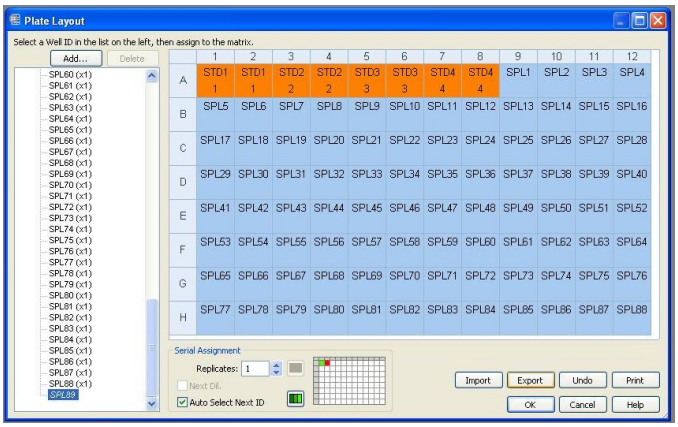


5d. Add the location of the standards and the samples to the 96-well plate. A button at the bottom (circled red here) can be switched between column and row entry (for this example, the button will have been clicked and then turned from left to right). To add the standards click on the first item under STD (or whatever you named it) (in this case "1", red arrow)on the left and then "paint" them into place. The cursor will look like a paintbrush. It will paint them in order, placing replicates next to each other (*i.e.*, STD1, STD1, STD2, STD2, etc.).



Repeat this for the Samples. Click on the sample name ID (SPL1 in this case) (green arrow). Then paint in the samples. You can click and drag to fill in samples from left to right. The software should name the sequentially (*i.e.*, SPL1, SPL2, SPL3, etc.). The finished plate should look something like you see to the right.

When finished, click "OK".



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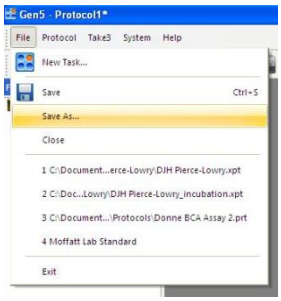
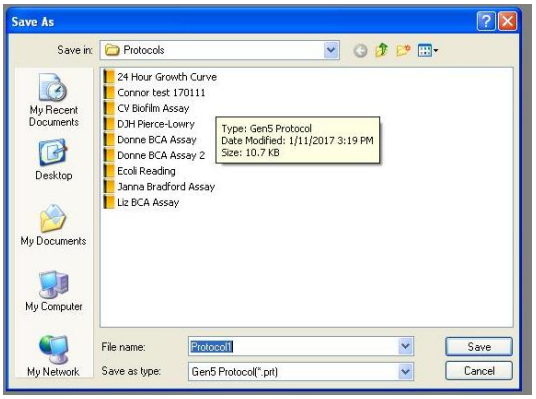
6. Save your protocol before running it. Click on the "File" menu and then select "Save as…".

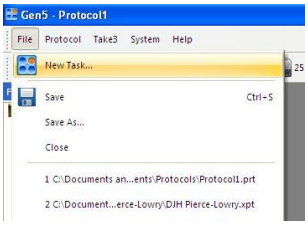
Select an appropriate folder and file name for your protocol.

Click "Save" when finished.

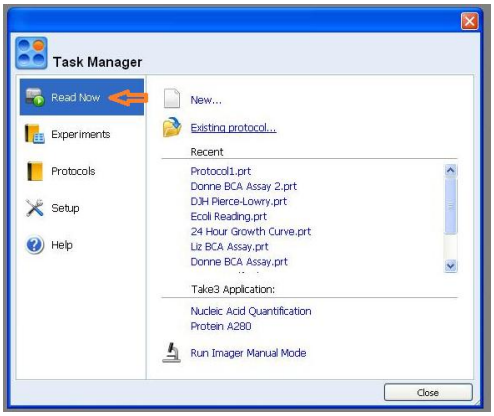




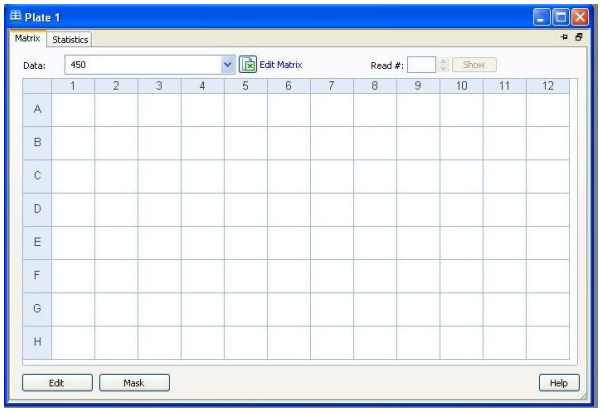
7. To run your protocol is moderately confusing. From the Gen5 software select under the "File" menu "New Task…", or click on the miniature "connect 4"-looking button.



You are now back on the Task Manager window, where you should click on "Read Now" at the left and then select your protocol from the right.



After you select your protocol, the procedure may actually auto-start, but will prompt you to put the plate in before proceeding. A window detailing the experiment results will come up first. If the experiment does not auto-start, or if you want to run additional measurements of the same procedure, click the green arrow button at the top.



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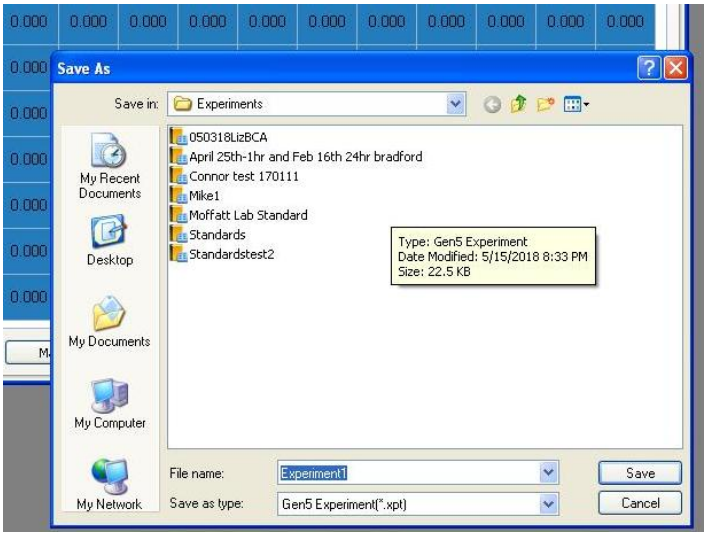
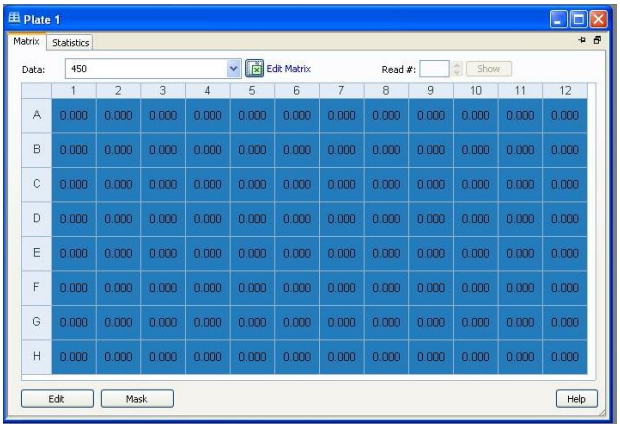
8. When the read is finished you should be prompted to save the results. If not, you can select "Save as…" from the "File" menu.

Save your results in your own folder on the computer.



8a. The results will be displayed on the screen as a value for each sample and standard, and may be color coded (you can set this up as an advanced feature). If you press the button that looks like the Excel logo, your results will be exported to an excel file. You can do further analysis there on your own.



Gen5 also has its own analysis features, which are not explored in this guide. To get to the analysis features, press the button in the top of the screen that looks like a calculator.