

## **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.

🐻 Read Now	Create New	
-	Open	
Experime to	Recent	
Protocols	Donne BCA Assay 2.prt	
	DJH Pierce-Lowry.prt	
Sot in	Ecoli Reading.prt	
	24 Hour Growth Curve.prt	
	Liz BCA Assay.prt	
🕐 Help	Donne BCA. Assay.prt	
1.1.1	Janna Bradford Assay.prt	
	CV Biofilm Assay.prt	
	Connor test 170111.prt	

My Computer (Computer (Computer (Computer (Computer) (Compute

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

reate New Protocol	
Select Protocol Type:	
💽 Standard Protocol	
Data reduction is performed indep	endently for each plate.
O Calibration Protocol	
Standards or Controls on the Calib analyze data on the 'Other' plates	rator plates (one or more) are used to
Calibrator plates:	1
Initial 'Other' plate count:	1
O Multi-Plate Assay Protocol	
Standards, Controls or samples are	e distributed across plates.
Number of plates:	1
All plates have identic	al layout
OUse several existing protocols on th	e same plate (paneling)
Do not show this dialog again	ប

Modified: 6/6/18



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".

Select steps	Plate Type:	96 WELL PLATE		V 🗌 Use lid
Actions		Cuvette		
Read Set Temperature	Select wells:	• Per step	O At runtime	
Shake Dispense	Description			Comments
Kinetic				
Start Kinetic Monitor Well Pause				
Delay Plate Out/In Stop/Resume Process Mode				
Well Mode Plate Mode Other				
Comment Options				



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:

Read Step							Read Step						
Step Label: Detection Method: Read Type: Read Speed:	Absorbance        Endpoint        Normal			Correction E	dit	Full Plate	Step Label:        Detection Method:     Fluorescence       Read Type:     Endpoint			Full Plate			
Wavelengths	01	⊙ 2 	03	04	05	O6	Filter Sets Excitation: Emission: Optics Position: Gain: I Filter Switching	0 1 360/40 460/40 Top 35 0ptions. per Well	<ul> <li>2</li> <li>360/40</li> <li>460/40</li> <li>Top</li> <li>35</li> <li>Options.</li> </ul>	○3 ▼ ▼	<u></u> 4	05	06
					ок	Cancel Help	Read Height:	1.00	mm		C	ок	ancel Help

Read Step						E
Step Label: Detection Method: Read Type: Integration Time:	<default> Luminescence Endpoint 1.0</default>	<b>S</b> 5. <i>s</i>				Full Plate
Filter Sets Excitation: Emission: Optics Position: Gain:	1 460/40 100 135 Options	<ul> <li>2</li> <li>460/40</li> <li>Top</li> <li>135</li> <li>Options</li> </ul>	O3	<b>O</b> 4	O5	06
Read Height:	per Well	nm		(	ок	Cancel Help

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



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.

Plate Layout Wizard	
Select well types	
Blanks	
Used for background signal subtraction	
Assay Controls	ntrole
Regacite, Posicite, Calibrators, or serially unded to	
Used for assay validation, cut-off analysis, normalization, or t curve comparison).	o generate reference curves (toxicology,
Number of different control typ	ies: 1
Standard Curves	
Required to generate standard curves and calculate unknown	concentrations.
Use multiple Standard Curves:	2
Samples	
Test wells requiring data analysis (calculation of concentration	, EC50,)
Sample Controls	
SPLC1 associated with Sample 1, SPLC2 associated w	ith Sample 2,
can be used as individual sample blanks, spikes	
	A Dark Next 2
Do not show wizard again	< DOLK NEXL > Cancel

late Layout ID: eplicates:	STD STD, REF	Full Name:	Standards, reference curve
Conc.\Dil. values	Colors		
🗹 Define diluti	ons/concentrations		
Type:	Concentrations	~	Unit:
STD1	1		
STD2	2		
STD3	3		
STD4	4		
STD5	· · · · ·		Auto
STD6			Increment:
STD7			
STD8			Factor:
STD9			
STD10			Clear list
		<u> </u>	CHOOL BOX



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.

ID Prefix: Replicates:	SF C	PL, UNK				
Default C	onc.\Dil. valu	s Identifica	ition Fields	Colors		
Type:	Dilutions		~		Unit:	
1				~		
2						
3				- Cital		
4					A	
5					AUCO	
6					Increment:	
7					-	
8					Factor:	
9						
10					Clear	list
11				(070)		

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".







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.

File	Protocol Take3 System Help	Save in:	C Protocols		• G	1 🕫 🗉	]-
	New Task Save Ctrl+S Save Atu Close 1 ChDocumenterce-Lowny.DJH Pierce-Lowny.incubation.npt 2 ChDocLownyiDJH Pierce-Lowny_incubation.npt 3 ChDocumentProtocoli/Donne BCA Assay 2.prt 4 Moffatt Lab Standard Exit	My Recent Documents Desktop My Documents	24 Hour Grow Cornor test 1 CV Biofilm Ass DHP Pierce-to Donne BCA As Donne BCA As Donne BCA Ass Ecol Reading Janna Bradfor Liz BCA Assay	h Gurve 70111 3V Type: GenS Protocol Date Modified: 1/11/20 say 2 Size: 10.7 KB	17 3:19 PM	]	
			File name:	Protocol1		~	Save
		Mu Network	Save as type:	Gen5 Protocol(* ort)		~	Cancel

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.



Help



🔡 Gen5 - Protocol1

New Task.

Save As Close

Save

File Protocol Take3

System Help

1 C:\Documents an...ents\Protocols\Proto

2 C:\Document...erce-Lown\DJH Pierce-Lowny.xpl

Ctrl+S

Close

Mask

Edit

E Plate 1

Data

A B C D E F G H 450



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.



⊞	Plate 1													
Ma	atrix	Statistics											-P 6	9
	Data:	450				- E	dit Matrix		Read a	#:	C Show	E		
1		1	2	3	4	5	6	7	8	9	10	11	12	
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	в	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	С	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	Е	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	н	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
(		Edit	Ma	ik 📄									Help	