BERGEN COUNTY | Technical Schools

Biotechnology Lab

BioTek Synergy HT

Multi-Mode Microplate Reader

Bergen County Technical Schools

Biotechnology Lab

Room 224A

BioTek Synergy HT Multi-Mode Microplate Reader Information Sheet

The BioTek Synergy HT Multi-Mode Microplate Reader is a single-channel absorbance, fluorescence, and luminescence microplate reader for research and development and *in vitro* diagnostic use. The instrument essentially functions as a spectrophotometer, which measures intensity as a function of the color, or more specifically, the wavelength of light. The instrument has applications that would provide useful for multiple disciplines, from Biology, to Chemistry and Nanotechnology.

The Synergy HT supports a wide range of labware, with many common microplate formats already preconfigured and ready for use in the software, ranging from 6 well to 384 well plates. .

The Gen5 Software configures and controls all measurement, protocols, and actions performed by the Synergy HT. The software supports detection for absorbance, luminescence, fluorescence intensity, fluorescence polarization, and time-resolved fluorescence (TRF) measurements. This instrument has been suggested to be used for studies involved with apoptosis, fluorescent molecule binding, cell proliferation and viability, immunoassays (such as ELISA).

The software allows the user to establish unique protocols to analyze different sample plates. Samples are analyzed in the instrument, and the results stored on the computer's hard drive. The data from the experiments are able to be viewed and analyzed in the Gen5 program, as well as in Excel by exporting the data.

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BioTek Synergy HT Multi-Mode Microplate Reader Quick Start Guide

1. Turn on the BioTek Synergy HT Multi-Mode Microplate Reader by flipping up the **Power Switch** on the front of the instrument.

   a. The light on the power switch will turn green.

   b. Allow the instrument to perform its start-up procedure before proceeding. When the instrument stops making noise, it is ready to use.

2. On the desktop, double click the **Gen5 Icon.**

Changing Filters

Cartridges containing the filters needed for all absorbance and fluorescence experiments should be left in the instrument. The filter cartridge only needs to be exchanged for luminescence experiments. Changing filters should only be done under the supervision of the instructor.

1. Open the **Filter Panel** on the front of the instrument (see Instrument Info Sheet).

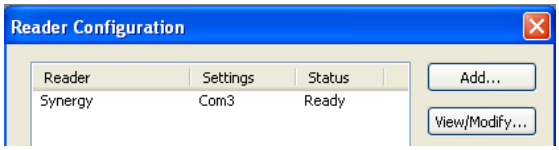
   Note: DO NOT open the panel or change filters if the power is on.

2. Unscrew the **Emission Filter Cartridge** and remove it from the instrument.

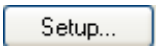
3. Take the **Blank Cartridge**, and insert the **Blocker Plug** into filter space 1. Lock it in place with the provided C-clip Filter Retainer (see Instrument Info Sheet).

4. Insert the blank cartridge with the blocker into the instrument, and tighten the screw to lock it in place.

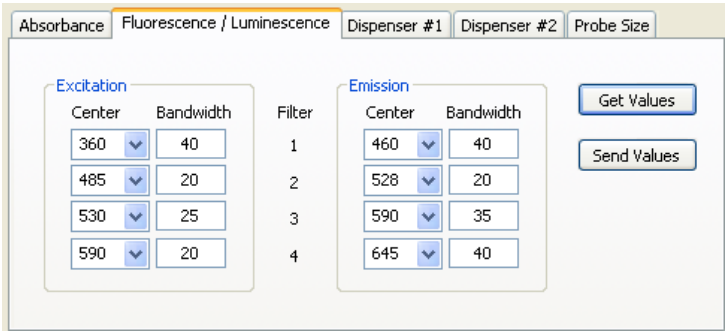
5. In Gen5, go to **System→Reader Configuration**, highlight the Synergy Reader, and click the **View/Modify Button**.



6. On the next screen, click the **Setup Button**.



7. Click the **Fluorescence / Luminescence Tab.**



   a. Write down the values that are found under **Emission**. These will need to be replaced after the protocol is run.

   b. Under **Center**, change the value of Filter 1 to **Plug**, and the rest to **Hole**.

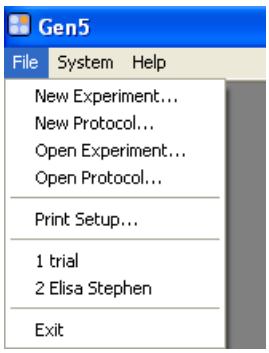
   c. Click **Send Values** to send the information to the instrument.

8. After the protocol is run on the microplates, and the instrument is shut down, remove the **Blank Cartridge** from the instrument and replace it with the **Emission Filter Cartridge**.

   Note: DO NOT open the panel or change filters if the power is on.

9. Repeat Steps 5 - 7 and replace the original values from the Emission Filter.

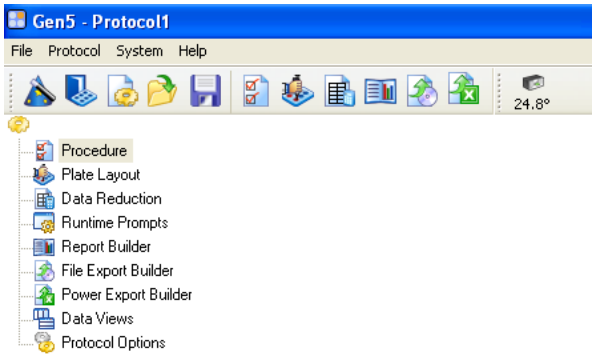
Setting up a Protocol



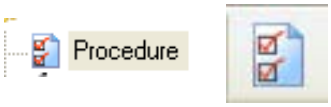
1. The Gen5 program will open to a blank screen. From this screen, click **File→ New Protocol** on the *Tool Bar* to set up a new protocol for an experiment.

   Note: If a protocol is already made for the experiment, proceed to the section **Running a Protocol on a Sample Plate.**

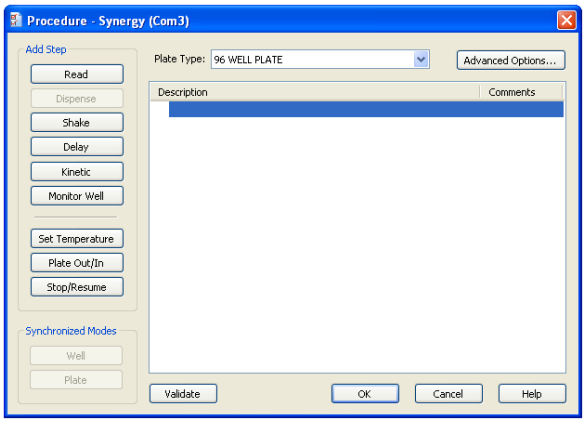
2. A screen will appear with icons along the *Tool Bar*, and a list of options along the left side.



3. Double click **Procedure** on the list, or click the **Procedure Icon** on the *Tool Bar.*



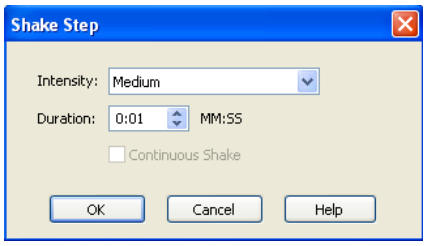
4. In the new screen, the procedure for the microplate will be created.



   a. Select **Plate Type** from the *Dropdown Menu*, and select the type of plate being used for the experiment.

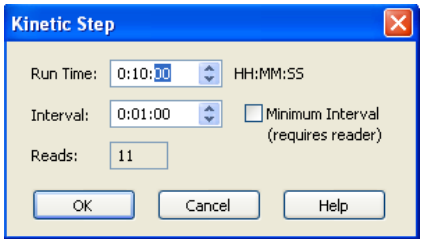
   b. Determine if the plate needs to be shaken prior to reading, if the temperature needs to be set for the instrument, or if multiple readings need to be taken over a period of time.

      i. Clicking the **Shake Button** brings up a screen that allows a shake step to be added. Click **OK**.

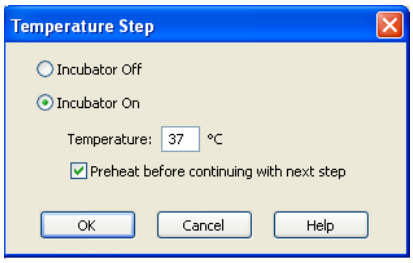


ii. Clicking the **Delay Button** delays the run prior to reading or performing another action. Click **OK.**

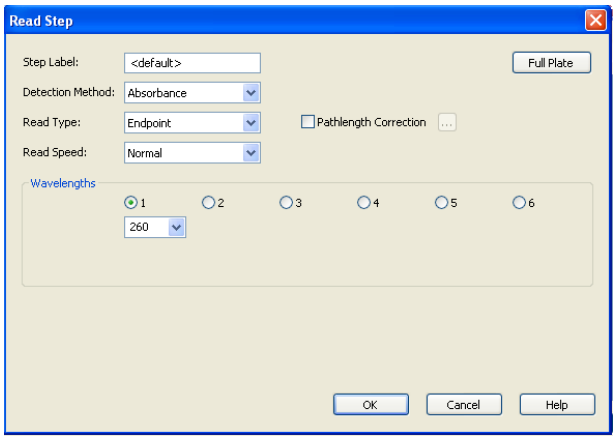
iii. Clicking the **Kinetic Button** allows multiple readings to be taken over a period of time (determined by the user). Click **OK.**



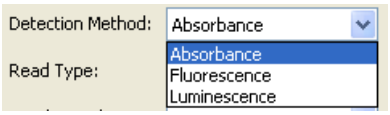
iv. Clicking the **Set Temperature Button** allows the internal temperature of the instrument to be adjusted for the read. Click **OK**.



c. Click the **Read Button** to enter the specific information about the type of read that is to be performed on the plate.



i. In **Detection Method**, select if Absorbance, Fluorescence, or Luminescence should be used on the plate.



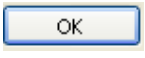
Note: The following options will vary slightly depending on the method that is chosen.

ii. Select the **Read type** that should be performed.

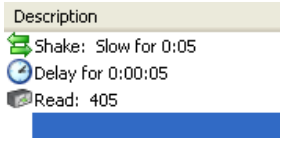
iii. Select the **Number of Wavelengths** or **Filter Sets** that should be used, and select the wavelengths to be used.

Note: Use this if the samples in the plate should be compared using two or more different absorbance or fluorescence settings.

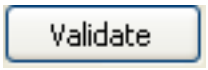
iv. Click **OK**.



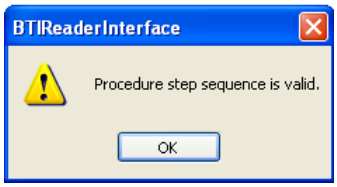
d. The steps may be reordered in the procedure by clicking and dragging one step above or below another step in the list.



e. To ensure that the instrument can perform the selected procedure, click **Validate**.

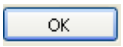


i. If the procedure is ok, the program says that the procedure is valid.

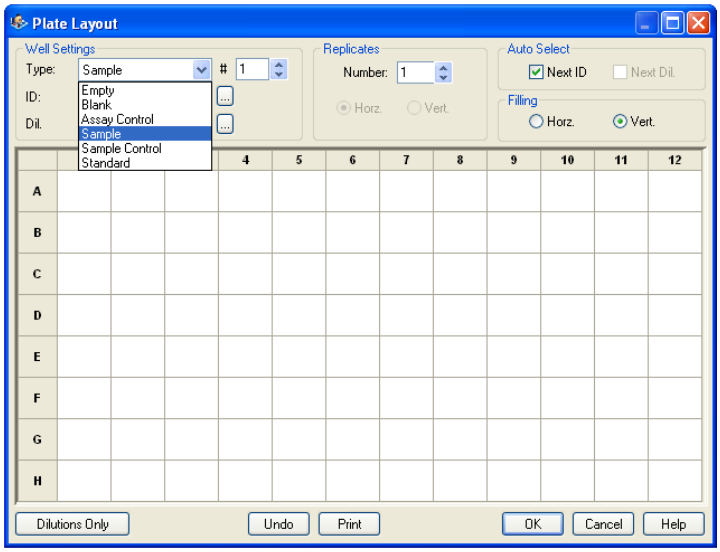


ii. If there is a problem with the order of the procedure, the program will direct the user to where to make changes.

f. Once the validation is ok, click the **OK Button**.

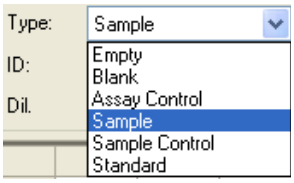


5. Double click **Plate Layout** on the list or click the **Plate Layout Button** on the *Tool Bar*.



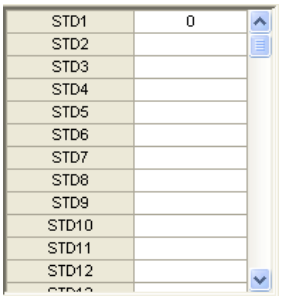
6. A new screen will pop up that will allow the wells in the plate to be defined.

a. First, select **Standard** from the *Type Dropdown Menu*.



b. Click the **(…)** button next to the **Concentration** area of the plate layout.

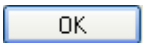
i. A screen pops up, and the concentrations of the standards can be entered in the appropriate boxes.



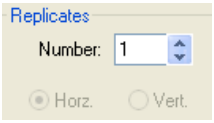
ii. Clicking the **Incr. Button** allows the concentration to be increased by the increment entered in the box, and added by clicking next to each standard.

iii. Clicking the **Fact. Button** allows the concentration to be increased buy a factor of whatever value is entered in the box, and added by clicking next to each standard.

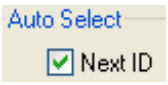
iv. Click **OK** once all standard concentrations are entered.



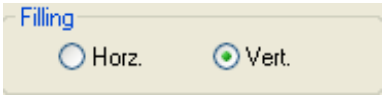
c. If there are replicates of the wells, select how many replicates there are in the **Replicates** box.



Note: The **Auto Select Button** will have Next ID selected. This causes the standard number to increase by 1 after each click on a well.

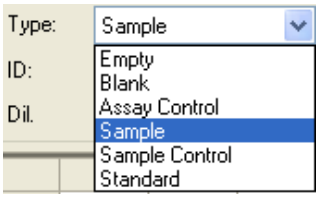


d. Select if the **Filling** should be **Vertical** or **Horizontal** in the plate.



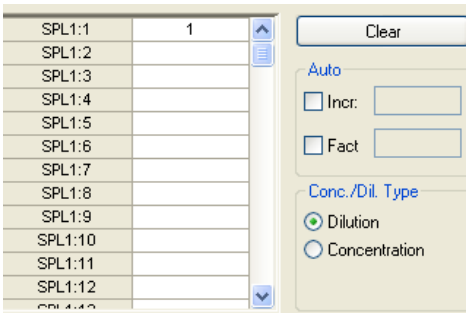
e. Click and drag the mouse across the cells that contain the standard. The cells will fill with the standard's number, and the concentration that was added.

f. Select the next type of wells to fill in the plate from the *Type Dropdown Menu*. Options are Sample, Empty, Blank, Sample Control, and Assay Control.

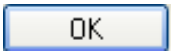


g. Repeat steps a – e for each type of sample or control until the entire plate is identified.

Note: For types such as Sample and the Controls, in addition to being able to enter a concentration by clicking the **(…) Button**, a **Dilution Value** can also be entered.



h. Once all the cells in the plate have been identified, click the **OK Button**.

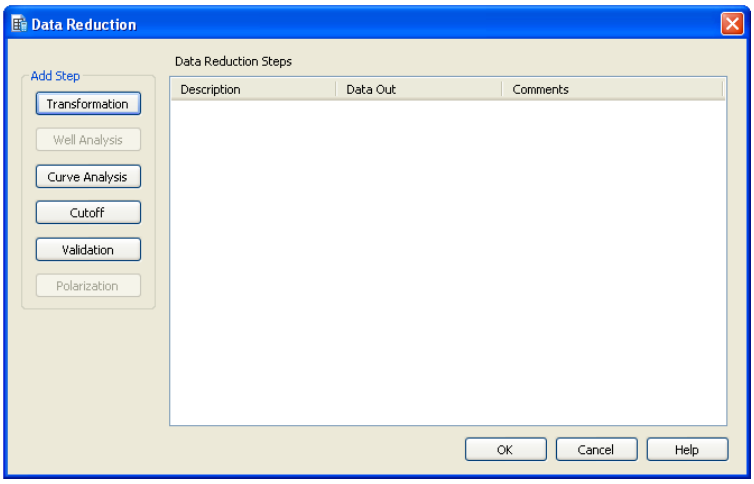


7. This is enough information to run the protocol. If no formulas are to be applied to the samples before the run, continue to 10, and then the section **Running a Protocol on a Sample Plate**.

8. To add formulas and calculations to the plates prior to the run, double click **Data Reduction** on the list, or click the **Data Reduction Button** on the *Tool Bar*.

Note: This is most useful when multiple wavelengths are being run on a plate, and when comparisons of the data from the different runs are to be analyzed after the run.

a. A window will pop up, with options for **Transformation**, **Curve Analysis**, **Cutoff**, and **Validation**.



b. Enter the appropriate formulas in the desired tabs, and then click **OK**.

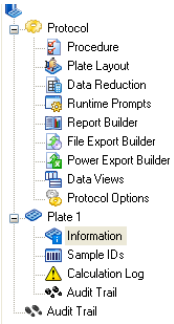
9. The protocol is now ready to be run on a plate. Click the **Save Button** on the *Tool Bar* to save the protocol.

a. Name the protocol with information such as the user's name, sample type, etc., and include the date the procedure was created.

Running a Protocol on a Sample Plate

1. Start a **New Experiment** by going to **File → New Experiment**, or by clicking the **New Experiment Button** on the *Tool Bar*.

2. Select the desired protocol from the list on the screen that pops up. Click **OK**.
3. A screen will appear with information similar to the procedure setup.



a. Verify the information in the protocol by clicking the **(+) Button** next to Protocol in the list on the left side of the screen. The Procedure and Plate Layout can be examined to ensure that all information is correct before running the plate.

4. To run the plate, click the **Read Plate Button** on the *Tool Bar*.

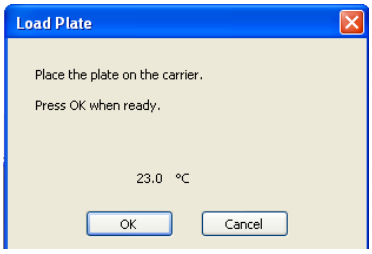
5. On the screen that appears, enter a **Plate ID**, or plate name, in the selected field.

    a. The plate name should identify the type of samples that are in the plate, or type of analysis being performed. The date should also be included.

6. Click the **Read Button** to read the plate

    a. The microplate carriage door will open. Place the microplate on the carriage with the letters to the left and the numbers at the top.

    b. A screen will pop up and instruct the user to click OK once the plate is loaded.

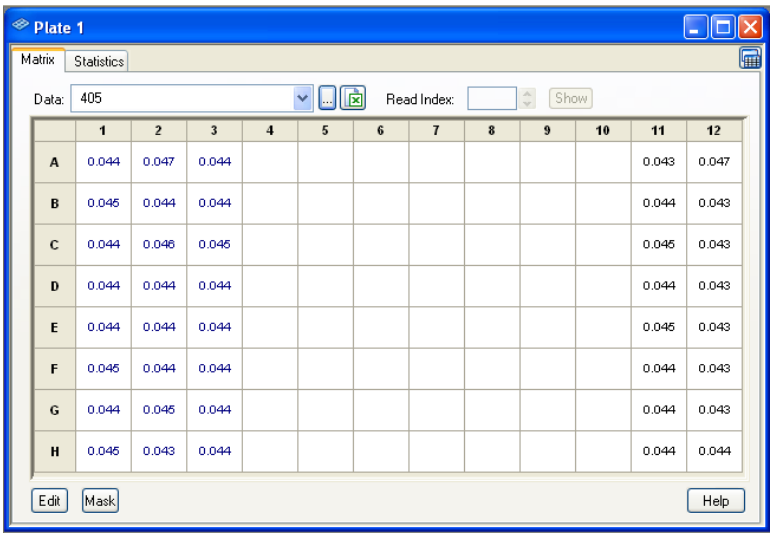


    c. The door will close, and the read will occur in the instrument according to the procedure that was created.

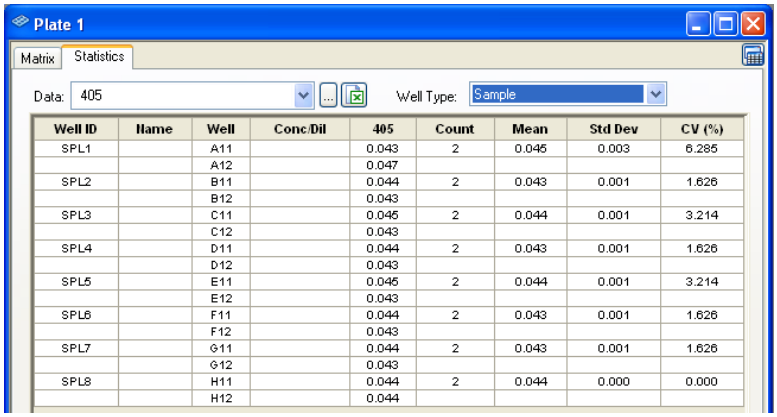
    d. When finished, the microplate will be ejected. Remove the microplate from the carriage, and press the **Close Door Button** on the instrument. The button should only be pressed once.

7. The reading from each well will appear on the screen in a microplate template.

    a. The **Matrix Tab** will show the reading for each individual well.



b. The **Statistics Tab** allows the data to be viewed according to the type of well.



i. This tab also gives the mean and standard deviation of each different sample if there were replicates selected in the plate.

8. The data can now be **Exported to Excel** by clicking the **Excel Button** in the *Plate Data Window*.

a. Clicking the **Excel Button** in the **Matrix Tab** will export the data in all the wells, and show the data in Excel in a format that looks like the microplate.

	A	B	C	D	E	F	G	H	I	J	K	L	M	
1		1	2	3	4	5	6	7	8	9	10	11	12	
2	A	0.044	0.047	0.044								0.043	0.047	405
3	B	0.045	0.044	0.044								0.044	0.043	405
4	C	0.044	0.046	0.045								0.045	0.043	405
5	D	0.044	0.044	0.044								0.044	0.043	405
6	E	0.044	0.044	0.044								0.045	0.043	405
7	F	0.045	0.044	0.044								0.044	0.043	405
8	G	0.044	0.045	0.044								0.044	0.043	405
9	H	0.045	0.043	0.044								0.044	0.044	405

b. Clicking the **Excel Button** in the **Statistics Tab** only exports the data on the screen. To export data from each of the different well types, the Excel button must be pressed when each one is selected on the screen.

	A	B	C	D	E	F	G	H	I
1	Well ID	Name	Well	Conc/DiI	405	Count	Mean	Std Dev	CV (%)
2	SPL1		A11		0.043	2	0.045	0.003	6.285
3			A12		0.047				
4	SPL2		B11		0.044	2	0.043	0.001	1.626
5			B12		0.043				
6	SPL3		C11		0.045	2	0.044	0.001	3.214
7			C12		0.043				
8	SPL4		D11		0.044	2	0.043	0.001	1.626
9			D12		0.043				
10	SPL5		E11		0.045	2	0.044	0.001	3.214
11			E12		0.043				
12	SPL6		F11		0.044	2	0.043	0.001	1.626
13			F12		0.043				
14	SPL7		G11		0.044	2	0.043	0.001	1.626
15			G12		0.043				
16	SPL8		H11		0.044	2	0.044	0	0
17			H12		0.044				

Saving Data and Shutting Down the Instrument

1. Once finished with the experiment and the data, click the **Save Button** on the *Tool Bar* to save the results of the run.

   a. Name the experiment and include the date that the plate was run. (Ex. StudentX_ELISAresults_4-29-09).

2. **Shut Down** the Gen5 program by clicking the **(X) Button** at the top right of the screen.

3. Make sure that the microplate was removed from the instrument, and turn the instrument off by pressing the **Power Switch** on the front of the instrument.

   a. The green light on the power button will turn off.

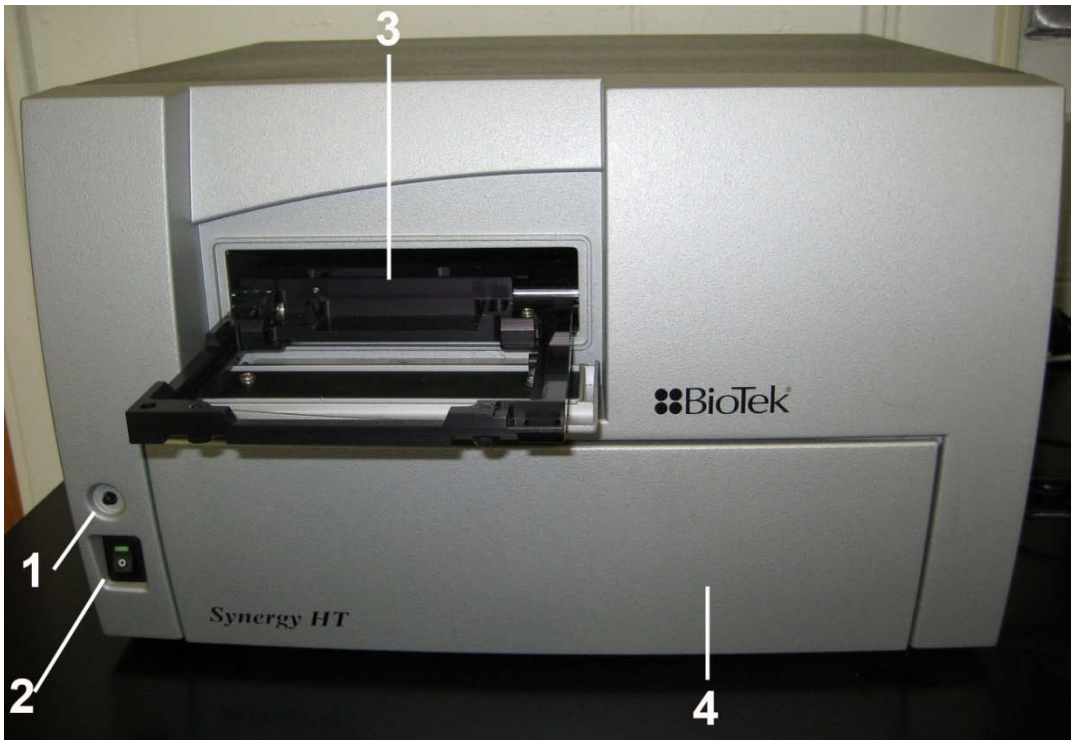
4. **Make sure the station is neat before leaving the instrument!**

BioTek Synergy HT Multi-Mode Microplate Reader Safety Sheet

1. Samples should be handled according to good laboratory procedures and methods in order to prevent accidents.
2. Dispose of all waste solutions according to waste disposal procedures.
3. Do not remove any panels or cords from the instrument to avoid electrical shock.
4. The instrument has moving parts:
    a. Do not attempt to exchange microplates or filters cartridges while the instrument is operating.
    b. Keep the work area around the instrument clear to avoid obstruction of the moving parts.
    c. When the microplate carriage is open, only place a microplate in the carriage to avoid breaking the instrument.
    d. Be aware of clothing and body parts when instrument doors are opening and closing.
5. Cleaning the workstation around the instrument is necessary. Never attempt to clean any internal spaces of the instrument.
6. Measurement values may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary for an accurate reading.
7. Food and drinks should not be placed on or near the instrument.
8. If any liquid should fall near the instrument, do not operate the instrument. Fluid seepage into internal components creates a potential shock hazard, and can cause the instrument to not work properly.
9. Running unauthorized programs or changing preferences on the instrument's computer is not allowed. Laptops are provided for personal use and internet access. Do not plug memory sticks in the instrument's computer unless instructed to do so by the instructor.

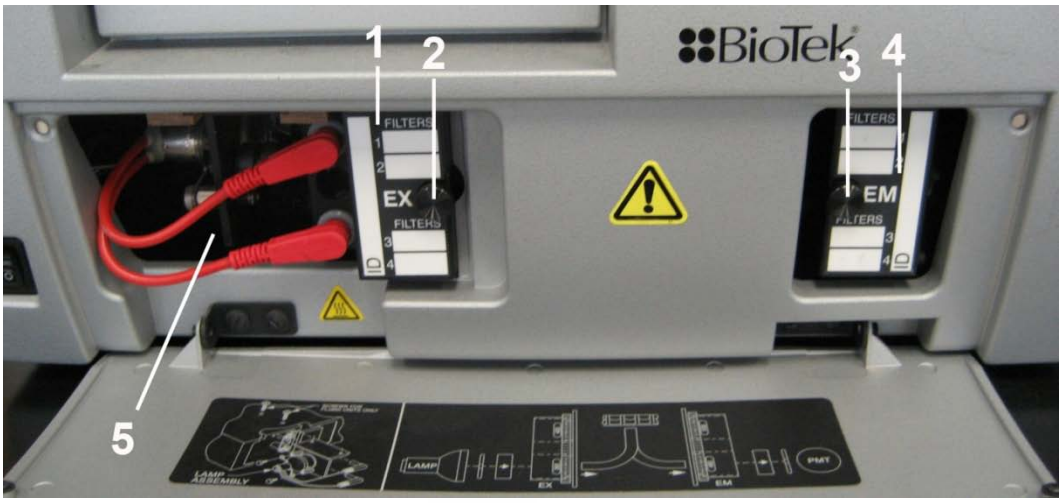
BioTek Synergy HT Multi-Mode Microplate Reader Instrument Information Sheet

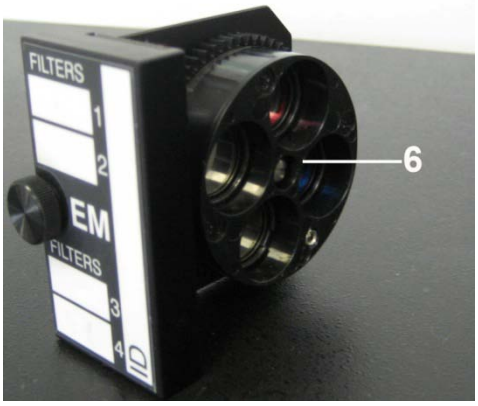
Synergy HT Multi-Mode Microplate Reader Overview:

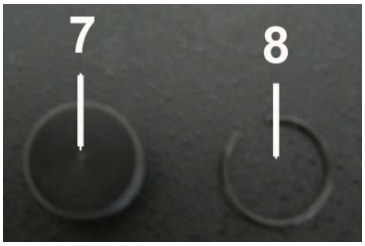


1. Microplate Carrier Eject Button
2. Power On / Off Switch
3. Microplate Carrier
4. Filter Panel Door (See next page)

Filter Panel Overview:







1. Excitation Filter Wheel Cartridge (Remains in instrument)
2. Excitation Filter Wheel Screw
3. Emission Filter Wheel Cartridge (Remains in instrument)
    i. Cartridge can be replaced with an empty cartridge containing 7 and 8.
4. Emission Filter Wheel Screw
5. Fluorescence Lamp Assembly
6. Filter Wheel with 4 filter openings
7. Blocker Plug for luminescence studies
8. C-clip Filter Retainer