



GFP Plate Reader Protocol



Theory and Introduction: Fluorescence detection - This method requires one wavelength to excite the molecule where a second longer wavelength is used to detect the fluorescence. The BioTek Synergy plate reader does this with a set of filters and fiber optics. The sample will be excited from above and detected from above or below. The biotek website is a great resource for fluorescence and for using this plate reader. The plate reader can work on a fixed wavelength, scanning UV/Vis, fluorescence, and as a luminometer. When using the plate reader to detect fluorescence, you must use the black plates. This helps to avoid light contamination and bleaching of sample in neighboring wells.

Sample Preparation: *a 96 well plate can hold up to 300 μ l. 50 to 100 μ l is typically sufficient. When conducting a MGH purification, transfer 100 μ l of each fraction or sample to a well. These samples can be returned to your tubes / samples.*

Protocol

1. Start up the instrument and after the machine stops making noise (1-2 min) then the PC. If the laptop is not on top of the instrument, then check the drawer under the reader. Connect the grey cord from the reader to the back of the PC.
 - username: chemistry - password: *blank* - just hit return
2. Get to the desktop and click on the KC4 shortcut icon.
3. For GFP quantization, choose the protocol menu and select GFP.prt (prt stands for protocol). If that protocol is not there, a flourDNAassay protocol will also be appropriate.
 - The excitation should be 485/20 nm and emission 528/20
4. On the top of the screen you should see: KC4 [new plate] [gfp.prt]
5. Click on the setting button. Under the plate type make certain that the "Greiner 96 flat bottom" is selected.
6. Check to see that top probe vertical offset is at 3mm.
7. Select OK
8. Click on the Layout button at the top of the screen.
9. Select the type of sample (control, blank, empty, sample, ect...) in the well settings as appropriate for your use.
10. The sensitivity will vary as the concentration of GFP in your samples change. Start with a sensitivity setting of 50. If the results are "stars" **** That means the value is too high. Adjust the sensitivity down. If the highest well has a number of less than 100-500 then adjust the value higher. If you have a sample you know to be fluorescent, use this in A1 and set that as the highest well to adjust to.
11. ID the wells with names as you see fit.
12. Hit OK
13. Skip the prompts and click on the "Start Reading" button.
14. Allow the instrument to warm up - it should only take 2 min.
15. Once the data is finished, click on export data to get an excel version of the report. Use the internet to email the report to you. If the internet connection is down, simply write the results in your book.
16. Remove the plate and shut the drawer by pushing the button in the front of the reader.
17. Shut off the machine (look at the arrow taped by the plate reader door)
18. Clean and return your plate.