

dsDNA Quantification

(Quantifluor™ Dye Systems; Promega # E2670)

1. Prepare standard curve:

| Standard | Volume of Lambda dsDNA Standard | Volume of 1X TE Buffer (μL) | Final Concentration of dsDNA (ng/μL) |
|----------|---------------------------------|-----------------------------|--------------------------------------|
| Blank | 0 | 1000 | 0.0 |
| A | 20 μL DNA Std + 980 μL 1X TE | 0 | 2.0 |
| B | 500 μL of Std A | 500 | 1.0 |
| C | 500 μL of Std B | 500 | 0.5 |
| D | 400 μL of Std C | 600 | 0.2 |
| E | 500 μL of Std D | 500 | 0.1 |
| F | 200 μL of Std E | 800 | 0.02 |

2. In a regular 96-well plate, prepare a 1:100 dilution of each unknown sample (2.5 μL sample + 250 μL AE Buffer).
3. In a black 96-well plate, transfer 100 μL of each standard and diluted sample *in duplicate* (use multi-channel pipette and Tip One filter tips to triturate 4x and transfer diluted samples to plate).
4. Prepare Quantifluor™ dsDNA Dye working solution: (10 μL 200X dye + 1,990 μL 1X TE) and add 100 μL per well (use multi-channel pipette and Tip One filter tips).
5. Incubate for approximately 5 min at RT, protected from light.
6. Measure fluorescence using the GMB Protocol on the BioTek SynergyMx 96-well plate fluorimeter. (Ex. 504; Em. 531).
 - a. Log onto computer.
 - b. Open Gen5 1.09 software.
 - c. Create New Expt
 - d. Select GMB_Quantifluor protocol
 - e. Maximize Protocol on left side of screen and adjust plate layout if necessary.
 - f. Under Plate at the top, select "Read".
 - g. Click the Read button.
 - h. Save file in GMB folder.
 - i. Insert plate according to instructions on screen.
 - j. Click OK.
 - k. After the machine finishes reading the plate, and data appears on the screen, click the "Statistics" Tab. For "Data", choose Conc x Dil.
 - l. Save as an excel spreadsheet by clicking on the excel icon button.
(R:\GMI\GMB\Data\QUANTIFLUOR)
 - m. Log off and close drawer (little black button).