

## DNA Quantification Using The Analyst Plate Reader and PicoGreen Reagent

This protocol describes one approach to fluorometric quantification of dsDNA samples using PicoGreen reagent (Invitrogen #P7581). It quantifies samples with starting concentrations between 0.01-10 ng/ $\mu$ l, and consumes 5 $\mu$ l per sample. The protocol and accompanying analysis spreadsheet can be modified depending on your exact needs.

*Pipette all standards and samples with care since the accuracy of your pipetting will limit the accuracy of your results!*

1. Prepare triplicate aliquots of each of the following dilutions of lambda DNA (Invitrogen #25250-010) in 1X TE buffer (10X TE = 100mM Tris pH8.0, 10mM EDTA pH8.0; sterile filtered if possible):

Standard 1 (1 ng/ $\mu$ l final conc. in assay):	8 $\mu$ l of 10 ng/ $\mu$ l lambda DNA
Standard 2 (100 pg/ $\mu$ l final conc. in assay):	2 $\mu$ l of Standard 1 plus 18 $\mu$ l 1X TE
Standard 3 (10 pg/ $\mu$ l final conc. in assay):	2 $\mu$ l of Standard 2 plus 18 $\mu$ l 1X TE
Standard 4 (1 pg/ $\mu$ l final conc. in assay):	2 $\mu$ l of Standard 3 plus 18 $\mu$ l 1X TE

Be sure to make the triplicate serial dilutions *separately* because the intent of the replicates is to average away any pipetting error. We have found that fluorescence increases linearly over the DNA concentration range represented by these standards. Higher concentrations give a non-linear response. Lower concentrations quickly exceed the sensitivity of the assay (i.e. signal is indistinguishable from background noise).

2. Working left to right along rows pipette 20 $\mu$ l of 1X TE into the first 12 wells (A1-A12) of a black 96-well plate (ISC BioExpress #T-3082-BLK). Pipette 5 $\mu$ l aliquots of standards 1,2,3 and 4 into wells A1, A2, A3 and A4 respectively, then repeat this pattern a second time in wells A5-A8 and a third time in wells A9-A12.

3. Pipette 20 $\mu$ l of 1X TE buffer into all of the remaining wells on the plate (or as many as you intend to use for samples) and add 5 $\mu$ l aliquots of each sample into consecutive wells, working left to right along each row. When you are finished, all wells will contain 25 $\mu$ l of liquid and the plate layout will be as shown below:

S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12
U13	U14	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24
U25	U26	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36
U37	U38	U39	U40	U41	U42	U43	U44	U45	U46	U47	U48
U49	U50	U51	U52	U53	U54	U55	U56	U57	U58	U59	U60
U61	U62	U63	U64	U65	U66	U67	U68	U69	U70	U71	U72
U73	U74	U75	U76	U77	U78	U79	U80	U81	U82	U83	U84

S1 = Standard 1

S2 = Standard 2

S3 = Standard 3

S4 = Standard 4

U1-U84 = unknowns (samples)

4. Turn on the Analyst plate reader (switch is on the right side toward the back) and wait for the drawer to open. Start the software by clicking the CriterionHost icon on the desktop (there is an instrument manual in the desk drawer below the machine).
5. At the CriterionHost main window you should see a green rectangle in the lower right corner indicating that communication with the instrument has been established. In the “Type” field select “Fluorescence Intensity” and then in the “Name” field select “DNA Tech Fluorescence Int”.
6. In the “Method” pull-down menu choose “Edit”. Check that the parameters are as shown below. In general these values shown should work well, but you can adjust them and re-scan the plate if you are not satisfied with the results. Information on the meaning of each parameter can be found in the help menu topic “Fluorescence Intensity method parameters”.

**Define and Edit Methods**

Luminescence | Multi-Method | Focused Luminescence | TRF  
 Absorbance | Epi-Absorbance | Fluorescence Intensity | Fluor. Polarization

Method Name: DNATech Fluorescence Int [Advanced]

**Optics**  
 Top  Bottom  
 Lamp: Continuous

**Filters**  
 Excitation: 1 Fluorescein 485 nm  
 Emission: 1 Fluorescein 530 nm

Plate Format: MJRBlackSkirt96 [Select wells]

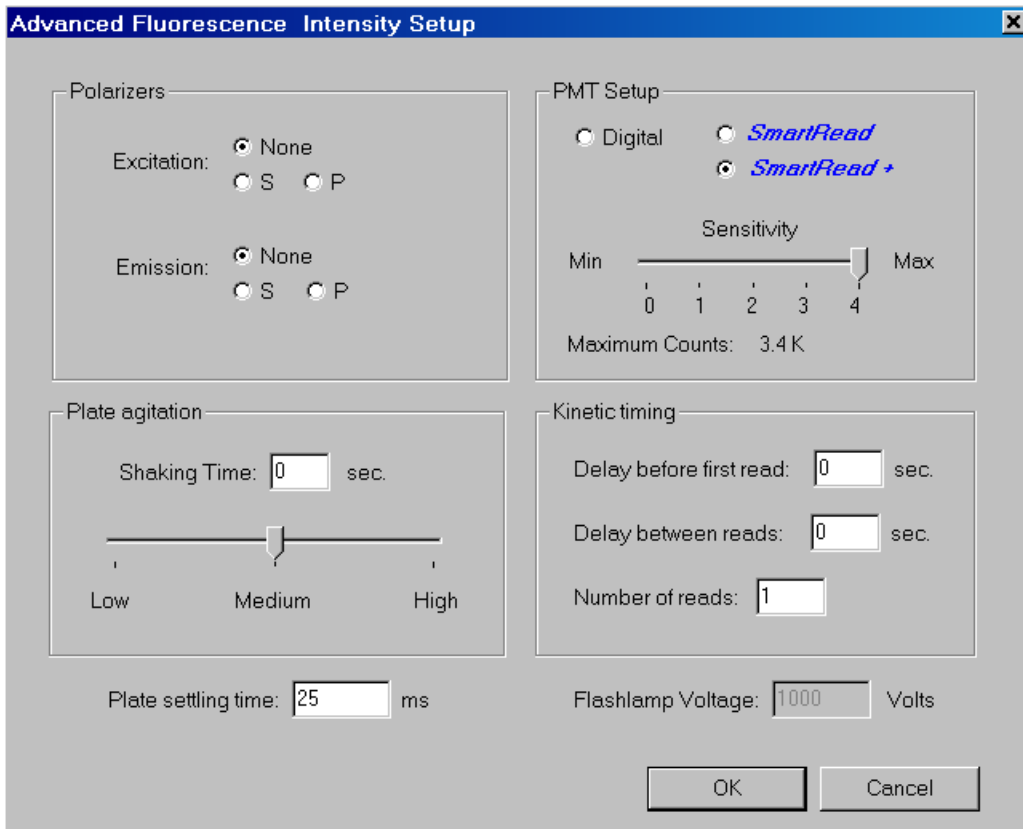
**Z Height**  
 Middle of well  Set 3 mm  
 Bottom of well

**Timing**  
 Readings per well: 1  
 Integration time: 100000  $\mu$ s  
 Interval between Flashes: 10 x 10ms  
 Delay after flash: 0  $\mu$ s

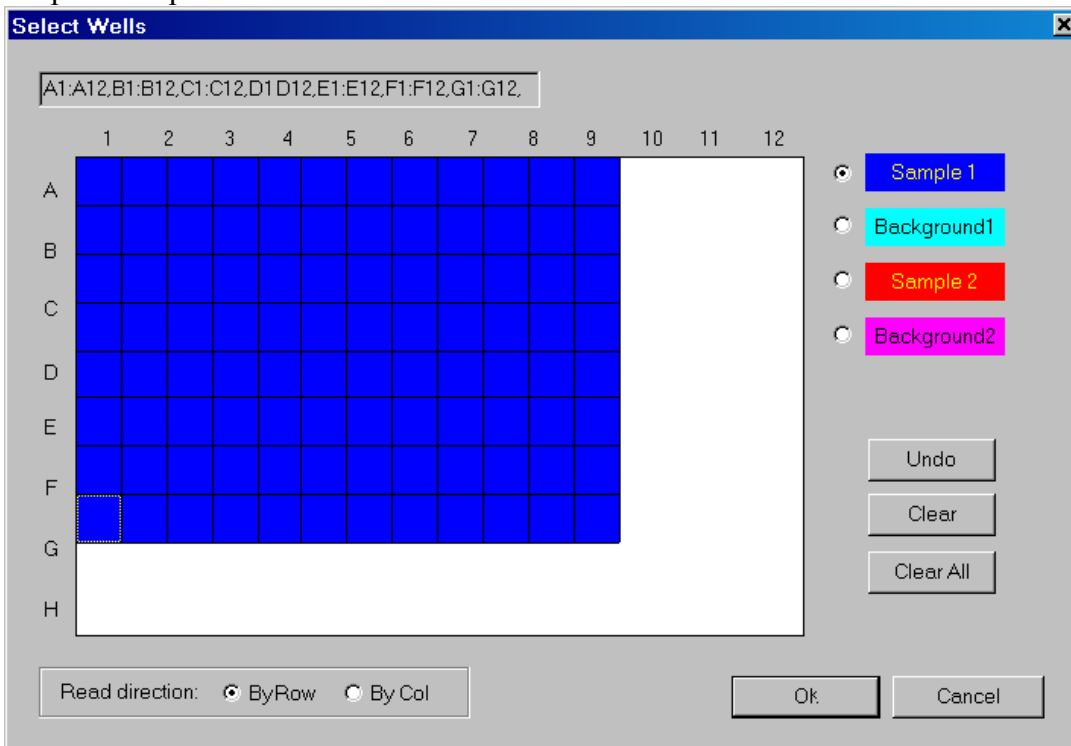
Raw Data Units: RFU Attenuator Mode: Medium

[OK] [Cancel] [Apply]

7. Click the “Advanced” button and check that the advanced parameters are as shown below. Again, these parameters should work fine but you can study their functions in the online help and change them if desired. When satisfied click “OK” to return to the main “Define and Edit Methods” window.



8. Click the “Select wells” button and use the mouse to select and color the wells of the plate template as shown below:



If you do not plan to use all of the wells you can leave the unused ones white. Click “OK” to return to the main “Define and Edit Methods” window, and then “OK” again to return to the CriterionHost main window. Note that due to a software bug the white grid overlaid on the plate map does not display at the correct scale. This does not affect assay performance.

9. Dilute PicoGreen reagent 100-fold in 1X TE. You will need 25 $\mu$ l per well plus 5% excess. For a full plate this works out to 25.2 $\mu$ l PicoGreen in 2494.8 $\mu$ l 1X TE. Mix thoroughly, and add 25 $\mu$ l to each well (use of a multi-channel pipetter speeds this step). Put the plate in the Lab-Line titer plate shaker at speed setting 7 and run for 5 minutes to thoroughly mix the contents of the wells and allow the dye to bind.

10. Remove the plate from the shaker and place it in the plate drawer of the Analyst plate reader with well A1 at the indicated position. Now click the Read button on the lower right side of the CriterionHost software window. The plate drawer will retract and the instrument will begin the read. It takes about a minute. The plate will eject again when reading is finished.

11. After reading the plate you can choose “Report view” from the “View” menu to see the raw data and the background-subtracted data. If you want to re-read the plate for any reason, just click “Read” and the new data will be appended to the bottom of the report (although you will not be able to see the previous reads until you open the report in Excel). Once you are satisfied with what you see use the “Save As” command from the “File” menu to save the data in your subfolder within C:/Analyst Data. Be sure to choose “Text files (\*.txt)” in the “Save as type” field of the Save dialog box.

12. Now start Excel (Start > Programs > Microsoft Excel) and open the report file you just saved (you will need to change the “Files of type” field in the Open dialog box to “Text Files (\*.prn; \*.txt; \*.csv)”. When the Text Import Wizard window opens, just click “Finish” to accept all defaults and import the tab-delimited file.

13. Open the file “PicoGreen Template.xls” in C:/Analyst Data and save a copy to your subfolder, giving it an appropriate new name (e.g. “mmddyPicoGreenAssay.xls”). Copy and paste the raw data from the CriterionHost report file you imported into the grid at the top of the PicoGreen Template (cells B3 through M10).

14. The standard curve should appear on the chart below the data. The four standards should all fall in a straight line. The equation of the best-fit line is given on the graph. If you have pipetted the standards carefully the  $R^2$  value should be quite close to 1.

15. The linear equation on the graph is in the form  $y=mx+b$ . Type the values of m and b into cells B35 and D35 respectively. Once these values are entered the concentrations of your unknowns will be calculated and displayed. Note that the FU value for each unknown must fall within the FU values of the highest and lowest standards in order for the concentration estimate to be considered reliable. Whether this is true for each unknown is indicated in the “Valid” column.

16. If desired you can type in sample names in cells B38 through B121. Selecting "Print" from the Excel "File" menu will print the completed results table. Be sure to save the updated file before exiting Excel.

17. Close Excel and CriterionHost when you are finished, then switch off the Analyst. Push the drawer closed after the machine is off (it's okay if it sticks out a little, and in fact pushing it in too far can cause problems for the next person). Leave the computer on.

18. We will transfer the excel files into your folder on the web server. The data can then be accessed by logging in to your account.