

Reproducibility and Linearity Assessment Using the Blue LED

Introduction

Two important parameters of quantitative steady state fluorometry are the reproducibility and the linearity of the detection system. This protocol will allow the user to test the sensitivity of the Thermo Scientific NanoDrop 3300 using the blue LED and also the linearity of the low range of the system.

Materials and Equipment

- NanoDrop™ 3300 Fluorospectrometer
- Fluorescein Cat# F36915 (Invitrogen Corp.)
- Borate buffer 50 mM pH 8.5 Cat# 28384 (Pierce Company)
- 1.5mL amber or foil covered snap top tubes, Fisher Cat# 05-408-134
- Vortex mixer
- Pipettes capable of delivering 2 uL, 20 uL and 1000 uL accurately

Protocol

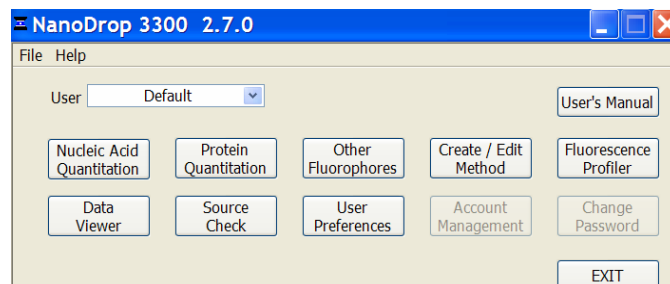
1. Prepare an initial serial dilution from a 50,000 nM fluorescein solution with 50 mM borate buffer as described in table 1 below:

Stock fluorescein [nM]	Fluorescein stock uL	Borate buffer uL	Total volume uL
50,000			60
5000	60	540	600
1000	160	640	800

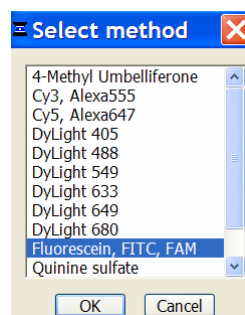
2. Prepare a working serial dilution from the 1000 nM fluorescein solution with 50 mM borate buffer as described in table 2. Use 1.5 mL snap top tubes and cover with aluminum foil.

Stock fluorescein [nM]	Fluorescein stock uL	Borate buffer uL	Total volume uL
1000			800
500	282	282	564
50	63.5	571.5	635
10	135	540	675
2.0	175	700	875
1.0	375	375	750
0.5	250	250	500
0	0	500	500

3. Launch the NanoDrop 3300 software and select the **Other Fluorophores** button.



4. Select the **Fluorescein, FITC, FAM** method and click OK:



5. Pipette 2 uL of deionized water on the lower pedestal and close the arm ensuring that the upper pedestal comes in contact with the water.
6. Wipe away water from both the upper and lower optical surfaces.
7. Pipette 2uL of the 50mM borate buffer on the lower pedestal, lower the arm, and click the **Blank** button.
8. Measure the serial dilution set from table 2 in triplicate, using a fresh aliquot for each replicate.
9. Statistics and R² values can be calculated on the data in the archive file when opened with Microsoft Excel.

NOTE: Typical performance of the NanoDrop 3300 fluorospectrometer is reproducibility of 10 %CV across the dilution set.

For Technical Support contact us at 302-479-7707 or nano-drop@thermofisher.com. Please include the archive file (C:\ND-3300 Data\User file\Application file) as an attachment when sending questions via email to the NanoDrop technical support team. For support outside of the US or Canada, please contact your local distributor.