Technical Note 179

Fluorescein Performance Data

Introduction

Fluorescein and its derivative fluorescein isothiocyanate (FITC) are ubiquitous in cell and molecular biology as a fluorescent label for cells and in many other applications. Fluorescein is a fluorescent compound that is easily detected using a fluorometer or fluorescence microscope. Fluorescein has an excitation maximum at 490 nm and an emission maximum at 514 nm.

DeNovix QFX Fluorometer and DS-11 Series Spectrophotometer / Fluorometers enable precise fluorescein quantification using a proprietary optical fluorescence core and four user-selectable LED channels. The data presented in this note was measured in the Basic Fluorometer app of EasyApps™ software.

Materials and Methods

A fluorescein dilution series diluted from a fluorescein standard (ThermoFisher Scientific cat #F36915) was quantified on a DeNovix QFX fluorometer.

The 50 µM standard was diluted in 0.05 M borate buffer with pH 8.5. A dilution series with 12 fluorescein concentrations between 0 nM and 2500 nM was created by serial dilution. Sample volumes of 200 µL were measured in 0.5 mL PCR tubes (DeNovix cat #TUBE-PCR-0.5-500). Measurements were made in the Basic Fluorometer app using the blue channel. The blue channel excitation range is 442 – 497 nm, and the emission range is 514 – 561 nm.

Results

The fluorescein standard curve from 0 to 500 nM is shown in Figure 1. Linearity was exhibited between 0.05 and 2500 nM fluorescein. Table 1 shows the raw data for the full dilution series.

Table 1: Fluorescein Raw Data through Full Concentration Range

Fluorescein Concentration (nM)	RFU (n = 3)	StDev	%CV
0	116.67	0.80	0.7
0.05	123.29	0.99	0.8
0.1	131.21	0.38	0.3
0.25	155.95	0.62	0.4
0.5	196.84	0.33	0.2
1.0	274.67	1.23	0.4
5	901.25	1.90	0.2
10	1699.35	3.65	0.2
25	4087.39	15.74	0.4
100	23394.95	33.14	0.1
500	113476.45	190.03	0.2
1000	221854.98	507.98	0.2
2500	534489.98	1459.92	0.3

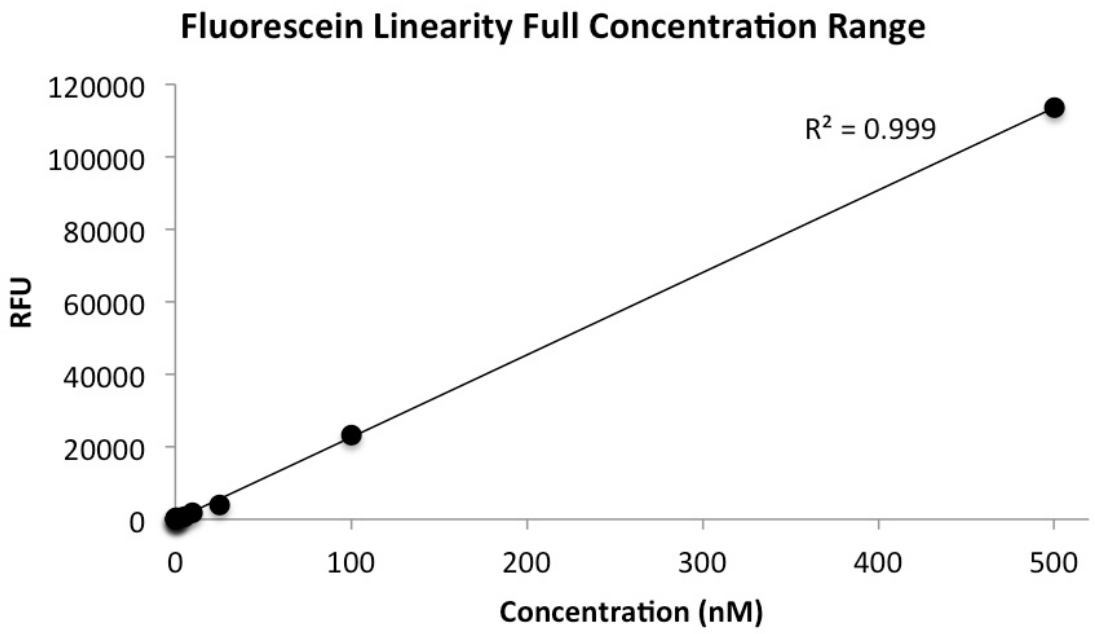

Figure 1: Fluorescein linearity through the concentration range of 0 – 500 nM.

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