



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.

The DeNovix® DS-11 Series Spectrophotometer / Fluorometer instruments enable precise fluorescein quantification using a proprietary optical core and four versatile fluorescence channels. DeNovix's preconfigured EasyApps® include fluorescence apps that enable the user to create additional assays for DNA, RNA, protein and other sample types. These apps include an LED Wizard in the assay creation window that helps the user select the appropriate fluorescence channel. The data presented in this note was measured in the Basic Fluorometer app.

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

Fluorescein Concentration (nM)	RFU (n = 3)	StDev	%CV
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2500	534489.98	1459.92	0.3
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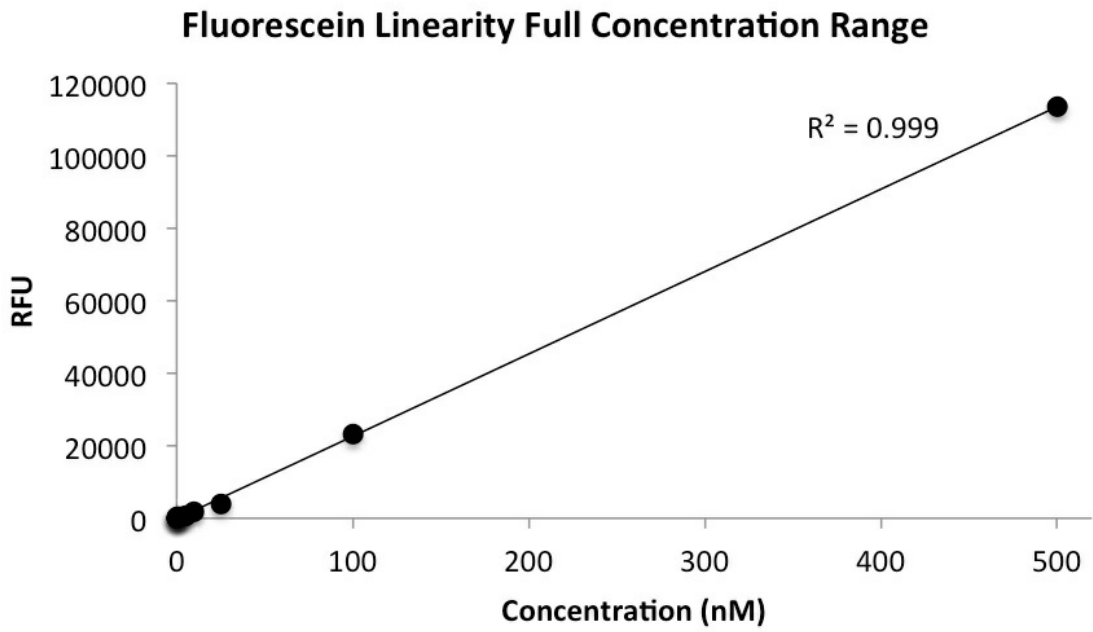


Figure 1: Fluorescein linearity through the concentration range of 0 – 500 nM.

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DeNovix Inc.
3411 Silverside Road
Wilmington, DE 19810, USA

Phone: +1.302-442-6911
Email: info@denovix.com
www.denovix.com

