Microvolume Alexa Fluor 555 Performance Data

Technical Note 115

Introduction

The DeNovix® DS-11 microvolume spectrophotometer SmartPath™ technology enables highly reproducible measurements of dyes often used to label probes for genomic and proteomic array studies. The Microarray and Labeled Protein apps allow the researcher to rapidly determine the post labeling efficiency of a reaction by determining both the concentration of a nucleic acid or protein and the conjugated dye in a single 1 µL sample.

The purpose of this technical bulletin is to present typical performance data demonstrating that the DS-11 can be used to determine the concentration of a dye across a broad linear range dye based upon the absorbance at its maximum excitation wavelength.

Materials and Methods

A 200 µM solution of Alexa Fluor® 555 carboxylic acid, succinimidyl ester (Life Technologies, #A20009) was volumetrically prepared in 0.05 M borate buffer. The buffer was prepared using a BupH Dry-Blend Pack (Pierce #28384) and HPLC grade water (Ricca #9153-1). A series of 10 dilutions ranging from ~0.4 µM to 200 µM was then prepared for the Alexa Fluor 555.

Reference concentrations for the dilutions were determined using an Agilent 8453 (Agilent, Santa Clara CA) in a 1 mm quartz cuvette (Starna, #1-Q-1).

The microvolume mode was used within the Microarray app on the DS-11 instrument to measure ten 1 µL aliquots for each dye solution. Fresh aliquots were used for each measurement.

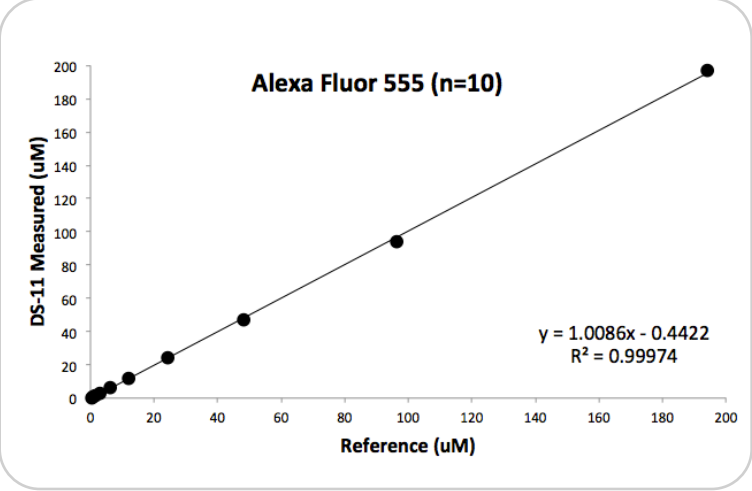
Concentrations were calculated by applying Beer's Law using 10 mm equivalent of the 555 nm absorbance values and an extinction coefficient of 1.5×10^5.

Results

The results presented below demonstrate that the DS-11 can be used to determine the concentration of a typical fluorescent dye used to label microarray and protein probes. The measurable concentration ranges for different dyes will be a function of the dye's extinction coefficient at the absorbance analysis wavelength.

DS-11 Typical Data for Alexa Fluor 555

Expected µM	Measured µM (avg. n=10)	Standard Deviation µM
0.00	-0.09	0.04
0.39	0.22	0.10
0.77	0.66	0.10
1.54	1.26	0.13
3.07	2.91	0.12
6.12	6.05	0.16
12.17	12.13	0.05
24.21	24.06	0.15
48.31	47.34	0.24
96.41	94.32	0.13
194.12	196.77	0.34



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