FITC Linearity, Reproducibility and Sensitivity

Introduction

Fluorescein is one of the most commonly used fluorophors in the research laboratory. In particular, the single-isomer fluoroscein isothiocyanate (FITC) is often used as a fluorescent label for immunoreagents and hybridization probes, and can be used as a tag to detect proteins. The Thermo Scientific NanoDrop 3300 Fluorospectrometer can measure as little as 1 ul of sample and thereby significantly scaling-down the reaction volumes commonly needed for conventional cuvette-based fluorometers. This micro-volume capability of the NanoDrop™ 3300 allows the researcher to run quality control checks for a variety of Fluorescein, FITC and FAM labeled reagents.

Method

Serially diluted fluorescein was measured using a 470 nm excitation source with the emission wavelength monitored at 515nm.

Results

Femtomoles of FITC per 2 ul measurement	FITC (nM)	Ave RFU (n=5)	Stdev	%CV
0.2	0.1	4	0.8	12.4
0.6	0.3	15	0.2	1.3
1	0.5	23	0.7	2.9
10	5	243	9.2	3.7
80	40	1364	60.1	4.4
400	200	6532	139.9	2.1
2000	1000	33163	1197.1	3.6
2500	1250	42109	857.7	2.0
5000	2500	82706	1246.6	1.5
10000	5000	160786	5320.9	3.3
20000	10000	314691	3779.9	1.2

Linearity was shown between 0.1 nM to 10000 nM.

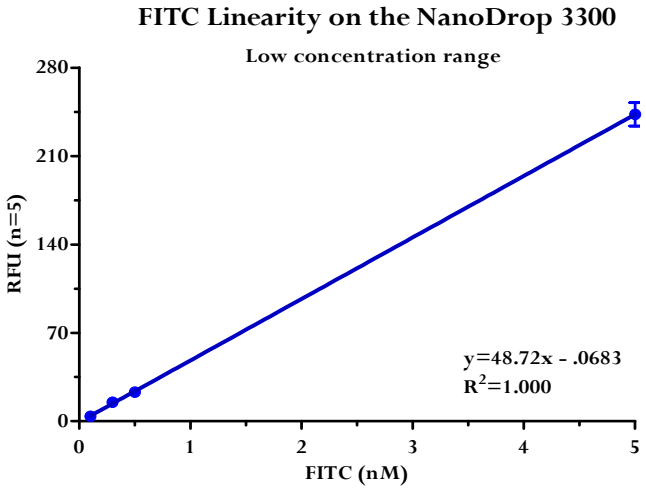

Figure 1: RFU values for 0.1 nM to 5 nM Fluorescein

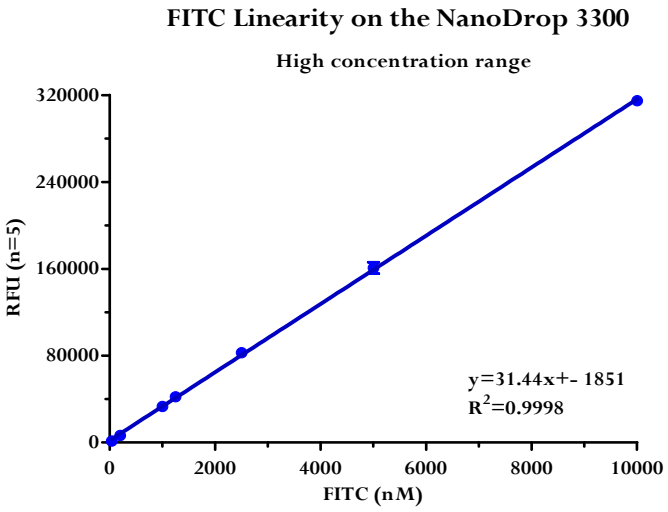

Figure 2: RFU values for 40 nM to 10,000 nM Fluorescein