Technical Note 171

ssDNA Assay Performance Data

Introduction

DeNovix DS-11 Series and QFX Fluorometers enable the accurate and specific quantification of nucleic acids, proteins and other biomolecules through fluorescence measurement. DeNovix Fluorometers utilize a proprietary optical core and a versatile set of four fluorescence channels for excitation and emission detection of fluorophores. Instruments include preinstalled EasyApps® configured for easy quantification using common ssDNA, dsDNA, RNA and protein fluorescence assays.

This document presents typical performance data measured on the DeNovix QFX Fluorometer using the Thermo Fisher Scientific Qubit® ssDNA Assay.

Materials and Methods

The Qubit® ssDNA Assay Kit (Thermo Fisher Scientific cat #Q10212) was used to perform the ssDNA assay. This assay quantifies ssDNA initial sample concentrations of 50 pg/µL – 200 ng/µL when using 1 – 20 µL of sample in 200 µL assay volume. The kit has a mix and measure protocol and includes buffer, reagent and two standards. Once prepared, it is strongly recommended to measure within 15 minutes for best results.

A series of dilutions was gravimetrically prepared in HPLC grade water from the Qubit® ssDNA Assay 20 ng/µL standard. For the assay, 10 – 20 µL of each prepared sample was added to 180 – 190 µL working solution. Initial sample concentrations were between 0.05 and 20 ng/µL.

Samples were measured in thin-walled, clear UV-transparent 0.5 mL PCR tubes (DeNovix cat #TUBE-PCR-0.5-500). Samples were mixed and incubated at room temperature for 5 minutes. Three replicate measurements were taken for each sample.

Performance Data

Performance data for the Qubit® ssDNA Quantification Assay was obtained on a Qubit® 3.0 and a DeNovix QFX Fluorometer. Table 1 below shows data for the Qubit® ssDNA Assay measured on both fluorometers. Expected concentrations were calculated based upon the gravimetric dilutions.

Table 1: ssDNA Assay Performance Data

ssDNA Assay				
Expected	Measured on QFX		Measured on Qubit®	
ng/µL	ng/µL	StDev	ng/µL	StDev
0.050	0.053	0.004	0.048	0.000
0.099	0.069	0.002	0.065	0.010
0.250	0.327	0.004	0.291	0.010
0.502	0.593	0.024	0.577	0.023
0.997	1.04	0.012	1.19	0.024
2.49	2.52	0.072	2.87	0.099
5.0	4.65	0.058	5.67	0.070
10	8.08	0.110	10.25	0.284
15	12.49	0.279	14.57	0.359

	ssDNA Assay			
20	20.43	0.139	18.46	0.225

Summary

The DeNovix Fluorometers enable sensitive quantitation of ssDNA when using the Qubit® ssDNA Assay. The data presented in this document shows that the DeNovix QFX ssDNA quantitation performance is equivalent to Qubit® performance when measuring ssDNA using the Qubit® ssDNA Assay.

Revised 19 Oct 2020

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