Technical Note 157

dsDNA Broad Range Assay Performance Data

Introduction

DeNovix Fluorescence Assays enable dsDNA quantitation over a broad range of concentrations through the use of three distinct fluorescence assay kits.

The DeNovix dsDNA Broad Range Assay is ideal for for measuring sample concentrations of 0.1 – 2000 ng/µL, with an upper extended range of 4000 ng/µL. The assay enables quantitation of dsDNA concentrations in the standard detection range when using 1 – 20 µL sample volumes in a 200 µL total assay volume. The upper detection limit can be extended by adding 1 µL of a 4000 ng/µL sample to 199 µL of reagent.

The assay dye has an excitation/emission maxima of 350/460 in the presence of dsDNA and is selective for dsDNA over RNA, ssDNA and protein. The assay is compatible with fluorescence microplate readers and fluorometers with the appropriate excitation sources and emission detectors. The excitation and emission spectra are shown in Figure 1.

This technical note presents typical performance data for the DeNovix dsDNA Broad Range Assay measured on a DeNovix DS-11 FX Fluorometer.

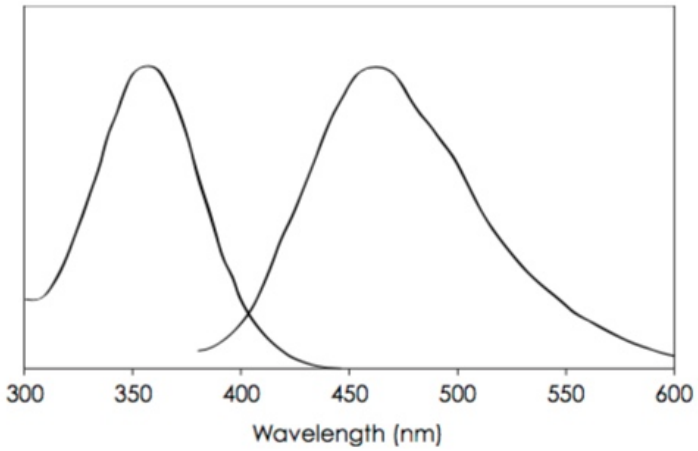

Figure 1: Excitation and emission spectra for the DeNovix dsDNA Broad Range Quantitation Reagent in the Presence of Excess dsDNA.

Materials and Methods

A series of dilutions of calf thymus dsDNA was prepared in TE buffer. The assay working solution was prepared by mixing 10 mL of the assay buffer with 100 µL of the dye and 100 µL of the assay enhancer solution.

For each sample, 190 µL of the working solution was added to a thin-walled, clear UV-transparent 0.5 mL PCR tube (DeNovix cat #TUBE-PCR-0.5-500). 10 µL of dsDNA was added to each tube for samples with concentrations between 0.5 and 200 ng/µL. For the lower concentration samples, volumes were adjusted to 180 µl working solution and 20 µL of dsDNA. For concentrations above 200 ng/µL, volumes used were 199 µL of working solution and 1 µL of sample.

Reaction solutions were mixed and incubated at room temperature for 5 minutes. Three replicates of each sample were then measured on three separate DeNovix DS-11 FX Fluorometers.

Linearity

The linear response of measured dsDNA as a function of expected concentration in the standard linear range is presented in Figure 2.

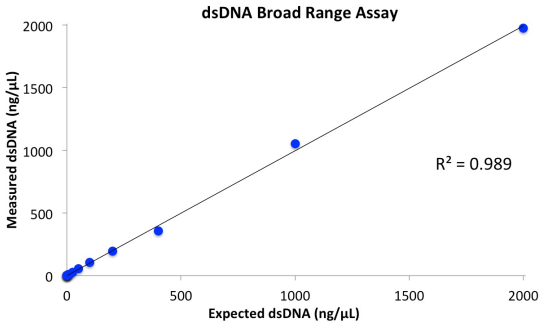

Figure 2: Average measured dsDNA across the standard range of the DeNovix dsDNA Broad Range Assay.

Performance Results

Concentration of dsDNA measured through the core linear and extended concentration ranges for the DeNovix Broad Range Assay are shown below in Table 1.

The data presented in Table 1 and graphically represented in Figure 2 demonstrate that the DeNovix dsDNA Broad Range Fluorescence Assay enables measurement of dsDNA through a range of 0.1 – 4000 ng/µL by varying mass and volume of samples.

Table 1: DeNovix dsDNA Broad Range Assay Results Measured on a DeNovix DS-11 FX

Expected dsDNA	**Measured dsDNA**	
ng/µL	ng/µL	StDev
0.1	0.116	0.005
0.2	0.237	0.011
0.5	0.542	0.0
1	1.12	0.015
2	2.39	0.011
6.25	6.65	0.020
12.5	13.48	0.017
25	26.83	0.030
50	53.75	0.051
100	106.1	0.094
200	195.7	0.274
400	358.08	0.263
1000	1050.92	0.071
2000	1974.53	0.134
3000	3123.24	0.248
4000	3551.61	0.353

Competitive Advantage

The DeNovix Broad Range Assay has a wider dynamic range than the comparable Thermo Fisher Qubit™ broad range assay. Figure 3 represents the high concentration range advantage of the DeNovix Broad Range Assay as compared to the Thermo Fisher Qubit™ assay.

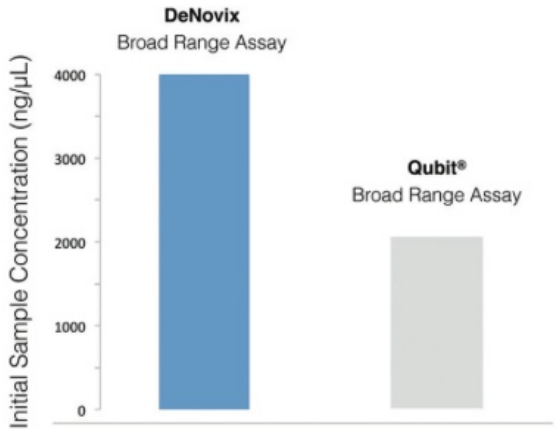

Figure 3: Comparison of the DeNovix dsDNA Broad Range Assay and the Thermo Fisher Qubit™ dsDNA Broad Range Assay.

Summary

The DeNovix dsDNA Broad Range Assay enables specific, highly sensitive dsDNA quantification across a wide dynamic range.

If the Broad Range Assay does not cover the concentration range of your samples, consider using one of the alternate DeNovix dsDNA Assay Kits listed in Table 2.

DeNovix Fluorescence Assay	Range
dsDNA Broad Range	0.1 – 2000 ng/µL
dsDNA High Sensitvity	5 pg/µL – 250 ng/µL
dsDNA Ultra High Sensitivity	0.5 – 300 pg/µL
RNA Assay	0.25 - 1500 ng/µL

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