



## Technical Note 159

### dsDNA Ultra-High Sensitivity 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 Ultra High Sensitivity Assay is optimized to enable the quantification of very low concentrations of dsDNA (0.5 – 300 pg/μL).

Like all the DeNovix dsDNA Assays, the Ultra High Sensitivity Assay selectively measures concentrations of dsDNA over RNA, ssDNA or protein. The assay dye is designed for use with fluorometers and fluorescence microplate readers. The DeNovix dye has an excitation maximum at 470 nm and emission maxima at 514 – 567 nm. The excitation and emission spectra are shown in Figure 1.

This technical note presents typical performance data for the measurement range of the DeNovix dsDNA Ultra High Sensitivity Assay measured on a DeNovix DS-11 FX.

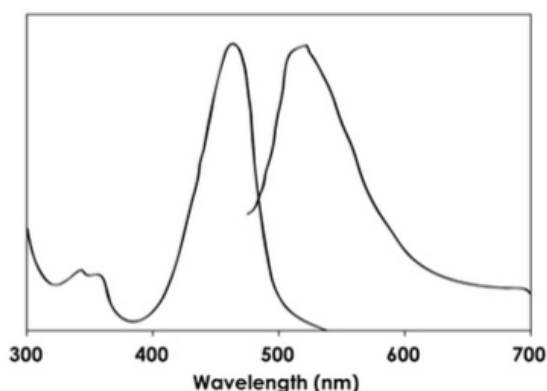


Figure 1: Excitation and emission spectra for the DeNovix dsDNA Ultra High Sensitivity Quantitation Reagent in the presence of excess dsDNA.

#### Materials and Methods

A dilution series of calf thymus dsDNA was prepared in TE buffer. The assay working solution was prepared by mixing 10 mL of the assay buffer with 25 μL of the dye and 100 μL of the assay enhancer solution.

For each sample, 200 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 300 pg/μL.

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 and expected concentration of dsDNA measured by the Ultra High Sensitivity Assay is presented below in Figure 2.

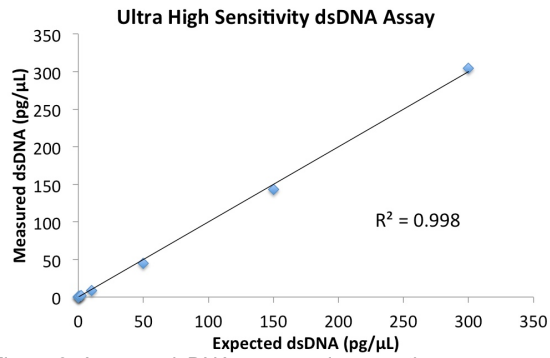


Figure 2: Average dsDNA measured across the concentration range of the DeNovix dsDNA Ultra High Sensitivity Fluorescence Assay.

### Performance Results

Concentration of dsDNA measured across the detection range for the DeNovix Ultra High Sensitivity Assay are shown in Table 1.

The data presented in Table 1 and graphically represented in Figure 2 demonstrate that the DeNovix dsDNA Ultra High Sensitivity Fluorescence Assay enables measurement of dsDNA through a range of 0.5 – 300 pg/μL.

Table 1: DeNovix dsDNA Ultra High Sensitivity Assay results, Measured on a DeNovix DS-11 FX

| Expected dsDNA | Measured dsDNA | StDev |
|----------------|----------------|-------|
| 0.5            | 0.503          | 0.031 |
| 1              | 1.189          | 0.104 |
| 2              | 1.832          | 0.041 |
| 10             | 8.519          | 0.09  |
| 50             | 45.18          | 0.18  |
| 150            | 142.91         | 0.26  |
| 300            | 304.4          | 0.33  |

### Competitive Advantage

As shown in Figure 3, the DeNovix dsDNA Ultra High Sensitivity Assay provides the user with a unique ultra low concentration sample assay option. Figure 3 below represents the high concentration range advantage of the DeNovix Ultra High Sensitivity Assay as compared to the Thermo Fisher Qubit™ assay.

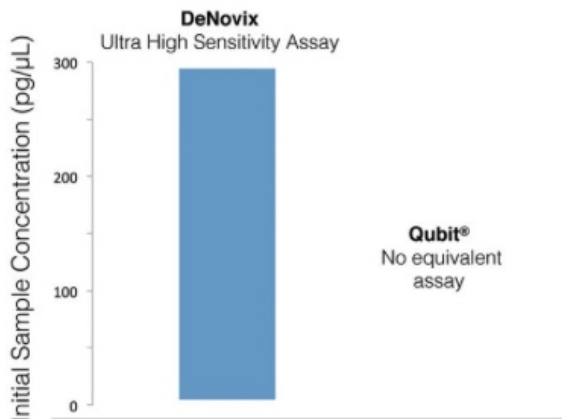


Figure 3: Dynamic range of DeNovix dsDNA Ultra High Sensitivity Assay.

### Summary

The DeNovix dsDNA Ultra High Sensitivity Assay enables accurate quantification of very low concentrations of dsDNA in an easy to use format. The simple mix and measure assay enables quantitation of dsDNA from 0.5 to 300 pg/μL.

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

| <b>DeNovix Fluorescence Assay</b> | <b>Range</b>        |
|-----------------------------------|---------------------|
| dsDNA Broad Range                 | 0.1 – 2000 ng/μL    |
| dsDNA High Sensitivity            | 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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