



Technical Note 207

DeNovix dsDNA High Sensitivity Assay Instructions for Thermo Fisher Qubit™ 3.0 / 4 Fluorometers

Introduction

The DeNovix dsDNA High Sensitivity Assay enables the accurate detection of purified double-stranded DNA (dsDNA) samples with a standard detection range of 100 pg – 250 ng total mass in 200 µL volumes. This equates to sample concentrations of 10 pg/µL – 250 ng/µL when using between 1 and 20 µL sample volumes in 200 µL assay volumes. The assay is linear for sample concentrations as high as 250 ng/µL when adjusting volumes to 1 µL of sample into 199 µL of working reagent. Total mass should not exceed 250 ng for best results.

The DeNovix High Sensitivity Assay is available for use on Thermo Fisher Qubit™ 3.0 and 4 fluorometers. The assay parameter files are [available here](#)

Kit Contents

Three assay sizes are available. The volume of components in each kit are sufficient for 1000, 250 and 50 (evaluation size) assays respectively. Kit components are shown in the table below. Safety data sheets are available at denovix.com/sds.

Component	1000	250	EVAL
DeNovix dsDNA High Sensitivity Dye (100x)	2 x 1 mL	0.5 mL	100 µL
DeNovix dsDNA High Sensitivity Buffer	200 mL	50 mL	10 mL
25 ng/µL dsDNA Standard (calf thymus)	2 mL	1 mL	0.5 mL
0 ng/µL dsDNA Standard	2 mL	1 mL	0.5 mL

Dynamic Range Considerations

The sensitivity of the Thermo Fisher Qubit™ 3.0 and Qubit™ 4 fluorometers decrease the dynamic range of DeNovix High Sensitivity Assay. For this reason, the 25 ng/µL standard must be diluted to 15 ng/µL when using the Thermo Fisher Qubit™ 3.0 or Qubit™ 4 fluorometers. The assay is linear on the Thermo Fisher Qubit™ 3.0 / 4 fluorometers as high as 150 ng/µL when adjusting sample volumes between 1 and 20 µL.

Installing DeNovix Assays on a Thermo Fisher Qubit™ Fluorometer

For a Thermo Fisher Qubit™ 3.0 / 4 fluorometer:

1. [Download](#) the available .qbt file.
2. Add the .qbt file to the root directory of a USB drive.
3. Insert the USB into a Thermo Fisher Qubit™ 3.0 / 4 fluorometer.
4. Navigate to Settings, and select Import Assay.
5. Select DeNovix High and choose the Thermo Fisher Qubit™ fluorometer folder where the assay will be stored. (It is recommended to store it under dsDNA).

Once the assay file is uploaded to the Thermo Fisher Qubit™ fluorometer, simply select the DeNovix High Sensitivity Assay to measure dsDNA samples.

Best Practices

- Use properly calibrated pipettes and DNase-free pipette tips.
- Treat all standards and samples identically in terms of incubation times and temperature.

- Generate a new standard curve for each assay.
- Ensure that sample solution contaminant levels are compatible with the assay.

Sample Prep

1. Allow all solutions to equilibrate to room temperature before use. Vortex, then centrifuge vials briefly before opening to minimize reagent loss on the cap.
2. Prepare working solution by mixing 10 mL of the assay buffer with 100 μ L of the dye. Scale volumes as needed to make enough volume to aliquot 190 μ L of the mixture for each standard and unknown. Discard after 24 hours.
3. For use on Thermo Fisher Qubit™ 3.0 / 4 fluorometers, dilute the 25 ng/ μ L standard by adding 6 μ L of the standard to 4 μ L of the assay buffer to make the 15 ng/ μ L standard.
4. For each standard or unknown sample, add 190 μ L of the working solution into a labeled tube. Adjust volume when adding more or less than 10 μ L of the unknown sample.
Note: Use thin-walled, clear 0.5 mL PCR tubes for assay measurements (DeNovix cat #TUBE-PCR-0.5-500 or equivalent). Label only the tops of the tubes.
5. Add 10 μ L of the 0 ng/ μ L, 15 ng/ μ L standards or 1 – 20 μ L of unknown DNA samples to the respective tubes and mix well. Avoid introducing air bubbles when mixing.
6. Incubate assay tubes at room temperature for 5 minutes. Protect from light.

Sample Measurements

1. Select the DeNovix High Sensitivity dsDNA Assay on a Thermo Fisher Qubit™ fluorometer.
2. Select the Read Standard option.
3. Insert the 0 ng/ μ L dsDNA standard tube, lower the lid and tap the Read Standard button.
4. Insert the second dsDNA standard tube, lower the lid and tap the Read Standard button
Note: For the Thermo Fisher Qubit™ 3.0 / 4 fluorometers, the standard is 15 ng/ μ L
5. After measuring the two standards, proceed to measure samples by tapping the Read Samples button.

Measurement Data

Three replicates of dsDNA samples were measured on a Thermo Fisher Qubit™ 3.0 fluorometer, a Qubit™ 4 fluorometer and a DeNovix QFX Fluorometer using the DeNovix High Sensitivity Assay. Each sample was prepared by adding 10 μ L stock to 190 μ L working solution. The data is presented in graphical and tabular form across the assay dynamic range below.

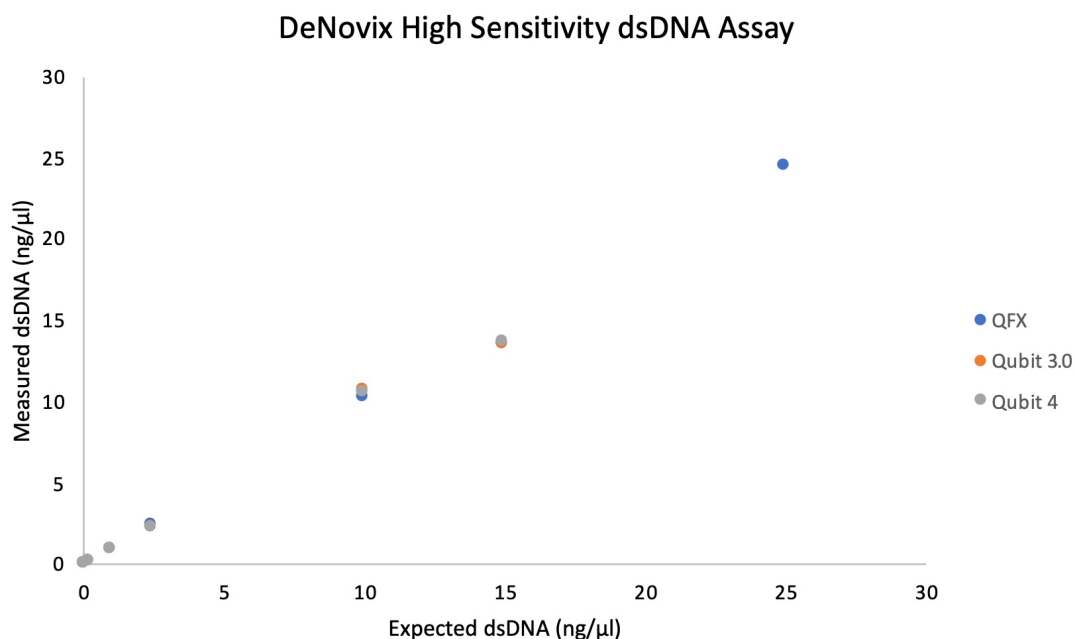


Figure 1: DeNovix High Sensitivity dsDNA Assay Linearity Data Measured on DeNovix QFX Fluorometer and Thermo Fisher Qubit™ Fluorometers

Table 2: DeNovix High Sensitivity dsDNA Assay Data Measured on DeNovix QFX Fluorometer and Thermo Fisher Qubit™ Fluorometers

Expected dsDNA	Thermo Fisher Qubit™ 3.0 Fluorometer		Thermo Fisher Qubit™ 4 Fluorometer		DeNovix QFX Fluorometer	
ng/ μ L	ng/ μ L	StDev	ng/ μ L	StDev	ng/ μ L	StDev
25	N/A	N/A	N/A	N/A	24.40	0.13
15	13.54	0.12	13.70	0.10	13.54	0.03
10	10.67	0.058	10.47	0.15	10.27	0.06

Expected dsDNA	Thermo Fisher Qubit™ 3.0 Fluorometer		Thermo Fisher Qubit™ 4 Fluorometer		DeNovix QFX Fluorometer	
2.5	2.22	0.00	2.29	0.012	2.34	0.01
1	0.91	0.01	0.92	0.008	0.91	0.009
0.25	0.20	0.001	0.21	0.002	0.21	0.004
0.1	0.08	0.00	0.08	0.002	0.08	0.001
0.03	0.02	0.001	0.02	0.001	0.02	0.000

Summary

The DeNovix dsDNA High Sensitivity Assay enables specific, highly sensitive dsDNA quantitation across a wide dynamic range. The assay is available for use on a Thermo Fisher Qubit™ fluorometer through the simple application of downloadable assay files.

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