

Introduction

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

The DeNovix High Sensitivity Assay is available for use on Qubit® 2.0 and 3.0 fluorometers. The assay parameter files are available on the DeNovix website.

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.

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

Dynamic Range Considerations

The sensitivity of the Qubit® 3.0 fluorometer decreases the dynamic range of DeNovix High Sensitivity Assay. For this reason, the 25 ng/ μ L standard must be diluted to 15 ng/ μ L when using the Qubit 3.0. The assay is linear on the Qubit® 3.0 as high as 150 ng/ μ L, when adjusting sample volumes between 1-20 μ L.

Installing DeNovix Assays on a Qubit® Fluorometer

For a Qubit® 2.0 fluorometer:

1. Download the appropriate .qbt file from the DeNovix website.
2. Add the .qbt file to the root directory of a USB drive. Ensure that this is the only .qbt file in the root directory.
3. Unplug the Qubit® 2.0, insert the USB, and turn the fluorometer back on by plugging it in.
4. Follow the prompts on the screen to download the assays.

For a Qubit® 3.0 fluorometer:

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

Once the assay file is uploaded to the Qubit® fluorometer, simply select the 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 sample solution contaminant levels are compatible with the assay.



Sample Prep

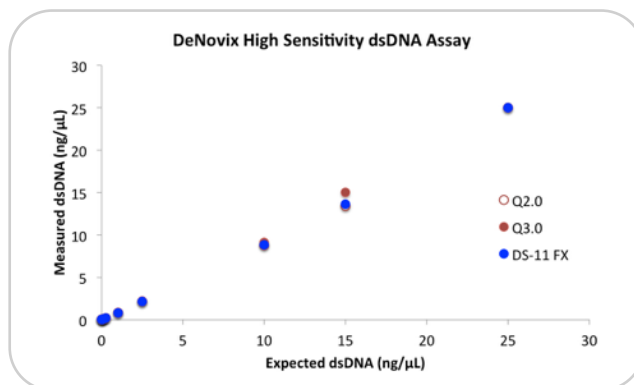
1. Allow all solutions to equilibrate to room temperature before use. Vortex, then centrifuge vials briefly before opening to minimize reagent loss on the cap.
2. Prepare working solution by mixing 10 mL of the assay buffer with 100 µL of the dye. Scale volumes as needed to make enough volume to aliquot 190 µL of the mixture for each standard and unknown. Discard after 24 hours.
3. For use on the Qubit® 3.0, dilute the 25 ng/µL standard by adding 6 µL of the standard to 4 µL of the assay buffer to make the 15 ng/µL standard.
4. For each standard or unknown sample, add 190 µL of the working solution into a labeled tube. Adjust volume when adding more or less than 10 µL of the unknown sample.
 - 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 µL of the 0 ng/µL, 25 ng/µL standards or 1-20 µL 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 Qubit® fluorometer.
 - On a Qubit® 2.0, the assay is added in a folder labeled DeNovix.
2. Select the "Read Standards" option.
3. Insert the 0 ng/µL 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.
 - For the Qubit® 3.0, the standard is 15 ng/µL, and for the Qubit® 2.0, the standard is 25 ng/µL.
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 Qubit® 2.0, Qubit® 3.0, and a DeNovix DS-11 FX fluorometer using the DeNovix High Sensitivity Assay. Each sample was prepared by adding 10 µL stock to 190 µL working solution. The data is presented in graphical and tabular form across the assay dynamic range below.



Expected dsDNA	Qubit® 2.0		Qubit® 3.0		DS-11 FX	
	ng/µL	StDev	ng/µL	StDev	ng/µL	StDev
25	25.00	0.000	N/A	N/A	25.00	0.012
15	13.40	0.000	15.00	0.058	13.62	0.005
10	8.76	0.072	9.12	0.020	8.81	0.020
2.5	2.13	0.012	2.21	0.012	2.14	0.003
1	0.82	0.002	0.84	0.002	0.80	0.002
0.25	0.21	0.002	0.21	0.002	0.20	0.001
0.1	0.09	0.001	0.08	0.003	0.08	0.001
0.03	0.03	0.001	0.02	0.002	0.03	0.001

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 Qubit® fluorometer through the simple application of downloadable assay files.

Qubit® is a registered trademark of Thermo Fisher Scientific and its subsidiaries.

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