



Technical Note 208

DeNovix dsDNA Ultra High Sensitivity Instructions for Qubit® 3.0 / 4 Fluorometers

Introduction

The DeNovix® dsDNA Ultra High Sensitivity Assay enables the accurate detection of purified double-stranded DNA (dsDNA) samples with a detection range of 5 pg – 3 ng total mass per assay tube. This is equivalent to sample concentrations of 0.5 – 300 pg/μL.

The DeNovix Ultra High Sensitivity Assay is available for use on Qubit® 3.0 and Qubit® 4 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.

Table 1: Ultra High Sensitivity Assay Kit Contents

Component	1000	250	EVAL
DeNovix dsDNA Ultra High Sensitivity Dye (400x)	0.5 mL	125 μL	25 μL
DeNovix dsDNA Ultra High Sensitivity Buffer	200 mL	50 mL	10 mL
DeNovix dsDNA B Ultra High Sensitivity Enhancer (100x)	2 x 1 mL	0.5 mL	100 μL
300 pg/μL dsDNA Standard (calf thymus)	2 mL	1 mL	0.5 mL
0 pg/μL dsDNA Standard	2 mL	1 mL	0.5 mL

Dynamic Range Considerations

The DeNovix Ultra High Sensitivity Assay enables dsDNA quantitation through the full dynamic range on Qubit® Fluorometers. However, the sensitivity of these instruments is inadequate to measure dsDNA at the low end of the assay range when following the standard protocol.

In order to measure dsDNA stock concentrations less than 10 pg/μL, sample volumes of 20 μL should be added to 190 μL working solution. For dsDNA concentrations above 10 pg/μL, the standard assay protocol is appropriate.

Installing DeNovix Assays on a Qubit® Fluorometer

For a Qubit® 3.0 / 4 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 / 4 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 DeNovix High Sensitivity Assay to measure dsDNA samples.

Best Practices

Pay careful attention to pipetting accuracy when quantitating low picogram amounts of dsDNA.

- Use properly calibrated pipettes and DNase-free pipette tips. Use the smallest calibrated pipettor available to dispense each sample volume.
- If sample dilutions are required, perform dilutions in the recommended assay tubes (DeNovix cat #TUBE-PCR-0.5-500 or equivalent).
- Prepare fresh working solution for each assay.
- Ensure that all samples and standards are treated identically in terms of incubation times and temperature.
- Generate a new standard curve for each 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 25 μ L of the dye and 100 μ L of the enhancer. Scale volumes as needed to make enough volume to aliquot 200 μ L of the mixture for each standard and unknown.
3. For each standard or unknown sample above 10 μ g/ μ L, add 200 μ L of the working solution into a labeled tube. For each unknown sample below 10 μ g/ μ L, add 190 μ L of the working solution.
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.
4. Add 10 μ L of the 0 μ g/ μ L, 300 μ g/ μ L standards or unknown DNA samples to the respective tubes and mix well. For unknown samples below 10 μ g/ μ L, add 20 μ L. Avoid introducing air bubbles when mixing.
5. 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.
2. Select the Read Standard option.
3. Insert the 0 μ g/ μ L dsDNA standard tube, lower the lid and tap the Read Standard button.
4. Insert the 300 μ g/ μ L dsDNA standard tube, lower the lid and tap the Read Standard button.
5. After measuring the two standards, proceed to measure samples by tapping the Read Samples button.
6. Indicate the volume of sample added into each tube (10 – 20 μ L).

Measurement Data

Three replicates of dsDNA samples were measured on a Qubit® 3.0, a Qubit® 4 and a DeNovix QFX Fluorometer using the DeNovix Ultra High Sensitivity Assay. Samples above 10 μ g/ μ L were prepared with 200 μ L working solution and 10 μ L sample. Samples below 10 μ g/ μ L were prepared with 190 μ L working solution and 20 μ L stock added. The data is presented in graphical and tabular form across the assay dynamic range below.

Table 2: DeNovix Ultra High Sensitivity dsDNA Assay Data Measured on QFX and Qubit® Fluorometers

Expected dsDNA pg/ μ L	Qubit® 3.0		Qubit® 4		QFX	
	pg/ μ L	StDev	pg/ μ L	StDev	pg/ μ L	StDev
300	300.7	1.242	297.3	1.155	302.8	2.402
150	157.3	0.577	156.3	1.155	158.1	0.145
50	47.07	0.115	47.73	0.231	48.72	0.757
10	7.77	0.178	8.31	0.155	9.39	1.55
2	1.20	0.150	1.37	0.108	3.10	0.242
1	Measured Out of Range		0.06	0.006	1.16	0.31
0.5	Measured Out of Range		Measured Out of Range		0.25	1.19

Summary

The DeNovix dsDNA Ultra 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.

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