Technical Note 201

DeNovix RNA Assay Instructions for Qubit® Fluorometers

Introduction

The DeNovix® RNA Assay enables the accurate detection of RNA samples with a standard detection range of 5 ng – 1500 ng total mass in 200 µL volumes. This equates to sample concentrations of 250 pg/µL – 1500 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 1500 ng/µL when adjusting volumes to 1 µL of sample into 199 µL of working reagent.

The DeNovix RNA Assay is available for use on Qubit® 2.0, 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 Table 1.

Table 1: DeNovix RNA Assay Kit Components

Component	1000	250	EVAL
DeNovix RNA Quantitation Dye (200x)	1 mL	250 µL	50 µL
DeNovix RNA Assay Buffer	250 mL	62.5 mL	12.5 mL
100 ng/µL RNA Standard (mammalian cell)	4 x 400 µL	400 µL	0.1 mL
0 ng/µL RNA Standard	2 mL	0.5 mL	0.5 mL

Best Practices

- Use properly calibrated pipettes and RNAse-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.

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.
   Note: The USB drive must have total storage capacity less than 2GB to be successfully read by the Qubit® 2.0 fluorometer.
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 Qubit® 3.0 and Qubit® 4 Fluorometers:

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 the assay and choose the Qubit® folder where the assay will be stored. (It is recommended to store it under RNA).

Once the assay file is uploaded to the Qubit® Fluorometer, simply select the DeNovix RNA Assay to measure RNA samples.

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 50 µL of the dye. Scale volumes as needed to make enough volume to aliquot 190 µL of the mixture for each standard and unknown.
3. 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.
   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 µL of the 0 ng/µL, 100 ng/µL standards or 1 – 20 µL of unknown RNA samples to the respective tubes and mix well. 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 RNA Assay on a Qubit® Fluorometer.
   Note: On a Qubit® 2.0, the assay is added in a folder labeled DeNovix.
2. Select the Read Standard option.
3. Insert the 0 ng/µL RNA standard tube, lower the lid and tap the Read Standard button.
4. Insert the 100 ng/µL RNA 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.

Measurement Data For Qubit® 2.0

Three replicates of RNA samples were measured on a Qubit® 2.0 and a DeNovix DS-11 FX Fluorometer using the DeNovix RNA Assay. Each sample was prepared by adding between 1-20 µL stock to 199-180 µL working solution respectively, depending on the total mass limits of the assay. The data is presented in graphical and tabular form across the assay dynamic range below.

Table 2: The data presented demonstrate the measurement range of the DeNovix RNA Fluorescence Assay measured on a DeNovix DS-11 FX Fluorometer and Qubit® 2.0.

Expected RNA	**Qubit® 2.0**		**DS-11 FX**	
ng/µL	ng/µL	StDev	ng/µL	StDev
0.5	0.58	0.005	0.48	0.014
1	1.00	0.006	0.83	0.006
2.5	2.60	0.015	2.39	0.024
5	4.60	0.125	4.49	0.035
10	10.63	0.058	11.18	0.130
25	25.23	0.115	26.89	0.583
50	48.27	0.153	52.05	0.230
100	98.50	1.249	98.34	0.177
1000	970.33	1.248	980.25	1.809
1500	Measured Out of Range		1530.39	22.029

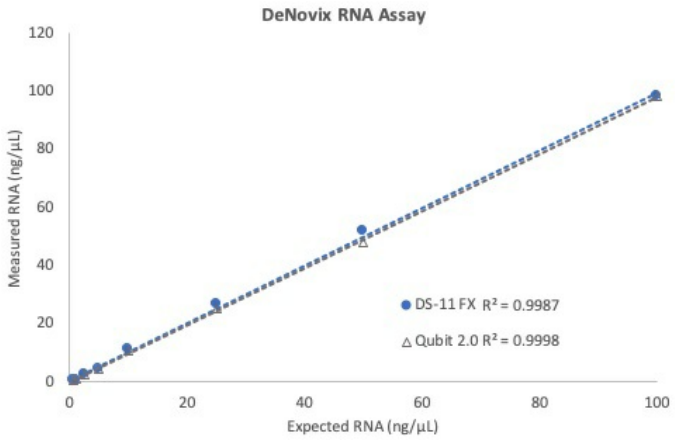

Figure 1: DeNovix RNA Fluorescence Assay results measured on a DeNovix DS-11 FX and Qubit® 2.0.

Measurement Data For Qubit® 3.0 / 4

Three replicates of RNA samples were measured on a Qubit® 3.0. a Qubit® 4, and a DeNovix DS-11 FX Fluorometer using the DeNovix RNA Assay. The data is presented in graphical and tabular form across the assay dynamic range below.

Expected RNA	**Qubit® 3.0**		**Qubit® 4**		**DS-11 FX**	
ng/µL	ng/µL	StDev	ng/µL	StDev	ng/µL	StDev
0.5	0.61	0.007	0.60	0.014	0.48	0.014
1	1.06	0.020	1.09	0.031	0.83	0.006
2.5	2.95	0.020	2.88	0.015	2.39	0.024
5	5.51	0.025	5.30	0.147	4.49	0.035
10	12.87	0.153	12.67	0.058	11.18	0.130
25	30.53	0.577	29.83	0.586	26.89	0.583
50	56.73	0.289	55.03	0.462	52.05	0.230
100	99.77	0.252	97.30	1.931	98.34	0.177
1000	997.33	0.305	973.33	0.208	980.25	1.809
1500	Measured Out of Range		Measured Out of Range		1530.39	22.029

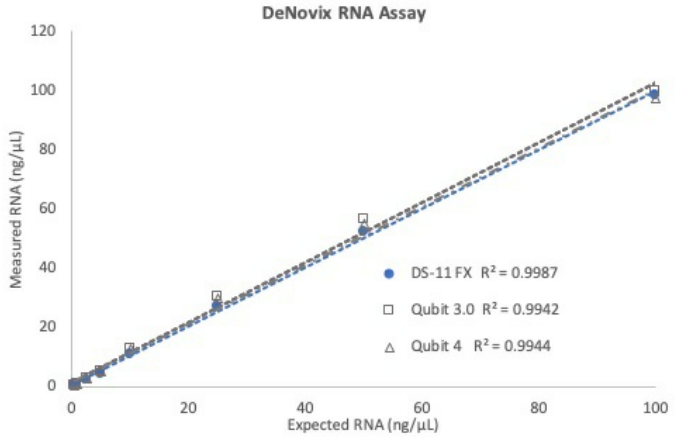

Figure 2: DeNovix RNA Fluorescence Assay measured results measured on a DeNovix DS-11 FX, Qubit® 3.0 and 4.

Summary

The DeNovix RNA Assay enables specific, highly sensitive RNA 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.

DeNovix Inc.
3411 Silverside Road
Wilmington, DE 19810, USA

Phone: +1.302-442-6911
Email: info@denovix.com
www.denovix.com