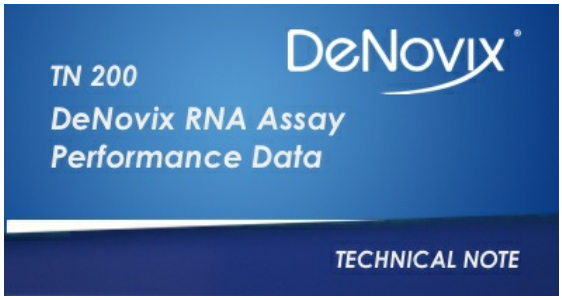

Technical Note 169

Qubit® RNA Assay Performance Data

Introduction

DeNovix DS-11 Series and QFX Fluorometers enable the accurate and specific quantification of nucleic acids, proteins and other biomolecules through fluorescence measurement. This note presents typical performance data measured on the DeNovix QFX using the Thermo Fisher Scientific Qubit® RNA High Sensitivity (HS) and Broad Range (BR) Assays.

The DeNovix Fluorometer utilizes a proprietary optical core and a versatile set of four fluorescence channels for excitation and emission detection of fluorophores. These channels enable fluorescent quantification of RNA, as well as dsDNA, ssDNA and protein. The DeNovix Fluorometer is preconfigured for these assays and automatically uses the correct excitation and emission filters.

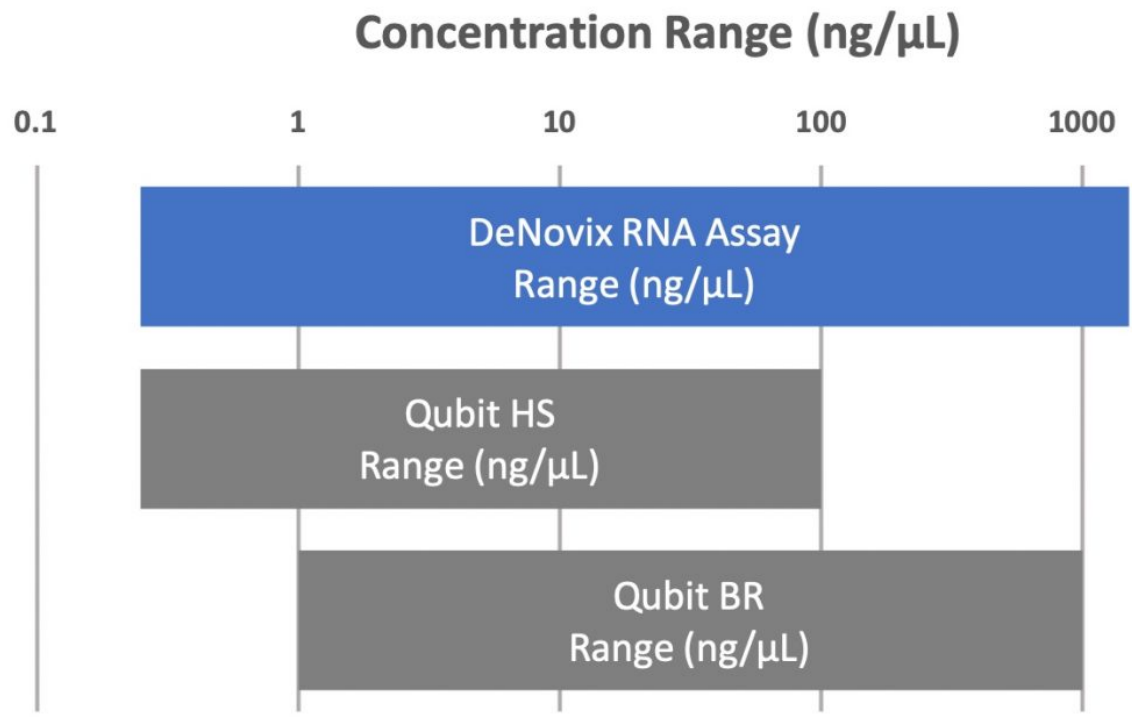

Figure 1. Dynamic Range □A comparison of Qubit® RNA and DeNovix RNA Assays.

RNA Quantification Assays

The Qubit® RNA HS Assay enables selective quantitation in the standard detection range of 5 – 100 ng total mass of RNA when using 1 – 20 µL in 200 µL assay volume. This equates to measuring RNA initial sample concentrations of 250 pg/µL – 100 ng/µL when using 1 – 20 µL of sample in 200 µL assay volume. The standard detection range of the Qubit® RNA BR assay is 20 – 1000 ng total mass when using 1 – 20 µL in 200 µL assay volume. This equates to measuring RNA initial sample concentrations of 1 – 1000 ng/µL when using 1 – 20 µL of sample in 200 µL assay volume.

Table 1: RNA HS Assay Performance Data

RNA HS Assay

Expected	Assay measured on QFX		Assay measured on Qubit®	
ng/µL	ng/µL	St Dev	ng/µL	St Dev
10	10.174	0.047	9.660	0.080
7.52	7.852	0.065	7.633	0.070
5.01	5.405	0.148	5.260	0.060
2.50	2.502	0.040	2.527	0.012
1.00	1.044	0.011	1.046	0.040
0.50	0.481	0.005	0.467	0.012
0.25	0.247	0.002	0.243	0.006

Table 2: RNA BR Assay Performance Data

RNA BR Assay

Expected	Assay measured on QFX		Assay measured on Qubit®	
ng/µL	ng/µL	St Dev	ng/µL	St Dev
100	102.636	0.865	98.200	1.400
75.03	81.598	0.675	76.200	0.600
50.06	53.915	0.186	51.733	0.416
24.96	26.451	0.133	25.667	0.115
10.66	10.098	0.096	10.167	0.058
5.14	4.943	0.145	4.820	0.040
2.50	2.650	0.075	2.513	0.012
1.00	1.120	0.005	0.873	0.006

Materials and Methods

The Qubit® RNA HS Assay Kit (Thermo Fisher Scientific cat #Q32852) and the Qubit® RNA BR Assay Kit (Thermo Fisher Scientific cat #Q10210) were used to perform both assays. Each kit has a mix-and-measure protocol and includes buffer, reagent and two standards. Samples were measured in thin-walled, clear UV-transparent 0.5 mL PCR tubes (DeNovix cat #TUBE-PCR-0.5-500).

Performance data for Qubit® RNA Quantification Assays were obtained on a Qubit® 3.0 and a DeNovix QFX Fluorometer. Each assay was prepared as described in the manufacturer's protocol. Samples were mixed and incubated at room temperature for 5 minutes. Three replicate measurements were taken for each sample.

A series of dilutions was gravimetrically prepared in HPLC grade water from the Qubit® HS and BR Assay standards. The samples for the HS assay were prepared from the 10 ng/µL standard, and the samples for the BR assay were diluted from the 100 ng/µL standard. Each sample was prepared to fall within the total mass limits of the respective assay. For each assay, 1 – 20 µL of each prepared sample was added to 180 – 190 µL working solution. Initial sample concentrations were between 0.25 and 10 ng/µL for the HS assay and between 1 and 100 ng/µL for the BR assay.

Summary

The data presented in this technical document shows that the DeNovix QFX Fluorometer enables sensitive quantitation of RNA when using a Qubit® RNA Assay. The DeNovix QFX RNA quantitation performance is equivalent to Qubit® performance when measuring RNA using both the Qubit® RNA HS and BR Assays.

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