

DeNovix RNA Assay Performance Data

Technical Note 200

Introduction

The DeNovix RNA Assay enables highly specific fluorescence quantification of RNA over a broad range of concentrations using a single assay.

The assay measures sample concentrations of 250 pg/ μ L to 1500 ng/ μ L RNA. This corresponds to a total mass of 0.5 ng to 1500 ng RNA.

The assay dye has excitation/emission maxima of 634/671 in the presence of RNA and is selective for RNA over dsDNA, ssDNA, and protein. The assay is compatible with fluorescence microplate readers and fluorometers with the appropriate excitation sources and emission detectors.

This technical note presents typical performance data for the DeNovix RNA Assay measured using the fluorometer mode of the DS-11 Series instruments.

Materials and Methods

DeNovix RNA Assay

A series of dilutions of *E. coli* total RNA was prepared in DEPC water. The assay working solution was prepared by mixing 4 mL of the assay buffer with 20 μ L of the dye. For each sample, 190 μ L of the working solution was added to a thin-walled, clear UV-transparent 0.5 mL PCR tube (DeNovix cat# TUBE-PCR-0.5-500). 10 μ L of RNA was added to each tube for samples with concentrations between 0.5 ng/ μ L to 100 ng/ μ L. For the samples with a concentration less than 0.5 ng/ μ L, volumes were adjusted to use 180 μ L working solution with 20 μ L of RNA. Measurements of concentrations above 100 ng/ μ L were made using volume adjustments so that the total mass did not exceed 1500 ng (e.g. 3 μ L of 500 ng/ μ L in 197 μ L of working solution).

Reaction solutions were mixed and incubated at room temperature for 10 minutes while protected from ambient light. Five replicate measurements of each sample were taken to assess linearity.

Materials and Methods (cont)

The linear response of measured RNA as a function of expected concentration in the full linear range is presented in Figure 1. The range of the assay can successfully be extended past both the upper and lower limits, and extension has impact on linearity.

Qubit® Broad Range (BR) and High Sensitivity (HS) RNA Assays

A Qubit® 4 fluorometer was used to test the same samples as the DeNovix RNA Assay with the Qubit® BR and HS kits to minimize dilution differences. The Qubit® BR kit (Q10210) (Lot# 2086147) was tested across the published range of 20 ng to 1000 ng. Likewise, the Qubit® HS kit (Q32852) (Lot# 2113240) was tested over the published range of 5 ng to 100 ng. All manufacturers instructions for sample handling were followed for each kit.

Comparison with Qubit® RNA Assays

The DeNovix RNA Assay covers a greater dynamic range than the combined range of the Qubit® BR RNA and Qubit® HS kits. Figure 1 compares the DeNovix RNA Assay dynamic range to the Qubit® BR and Qubit® HS RNA assays.

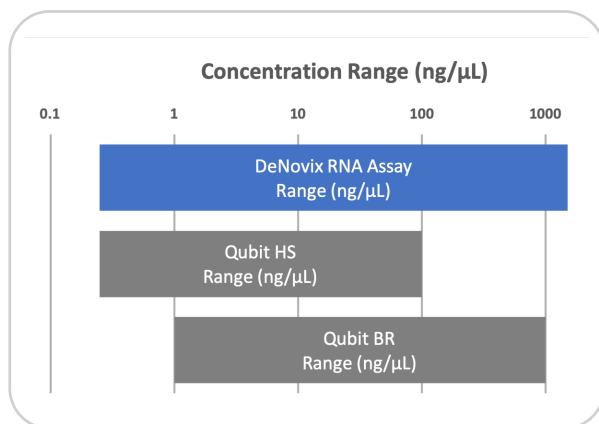


Figure 1. Dynamic Range

A comparison of Qubit® HS RNA and DeNovix RNA Assays.



Performance Results

Figures 2 and 3 below demonstrate that the DeNovix RNA Assay enables measurement of RNA through a range of 0.5 ng (20 μ L of 0.25 ng/ μ L) to 1500 ng (1 μ L of 1500 ng/ μ L) by varying mass and volume of samples. The assay performance is linear over the range analyzed with an exceptional R^2 value.

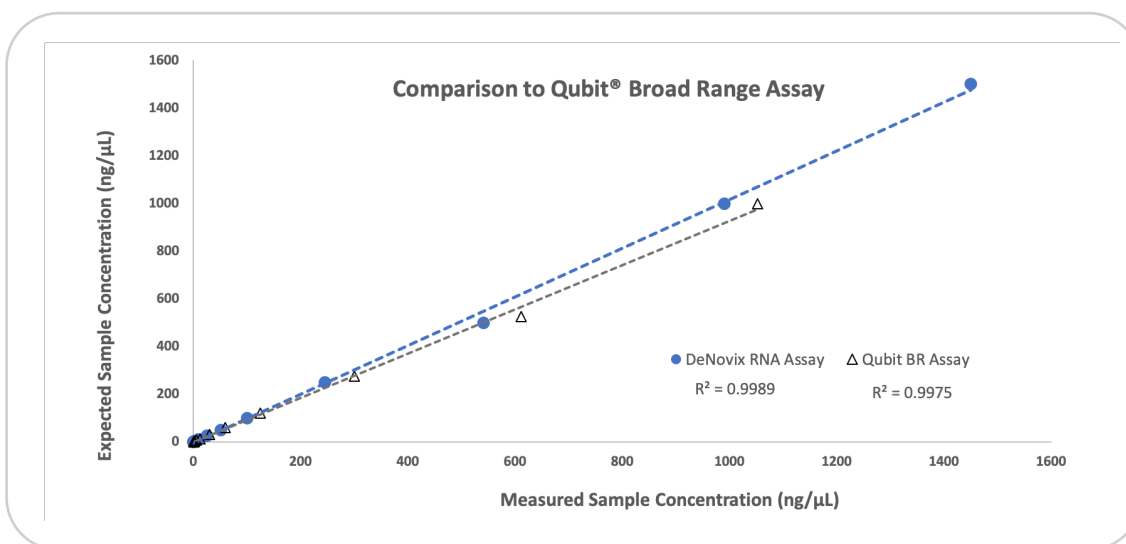


Figure 2. Comparison of Qubit® BR RNA and DeNovix RNA Assay

A comparison of the Qubit® BR RNA and DeNovix RNA Assay including linearity.

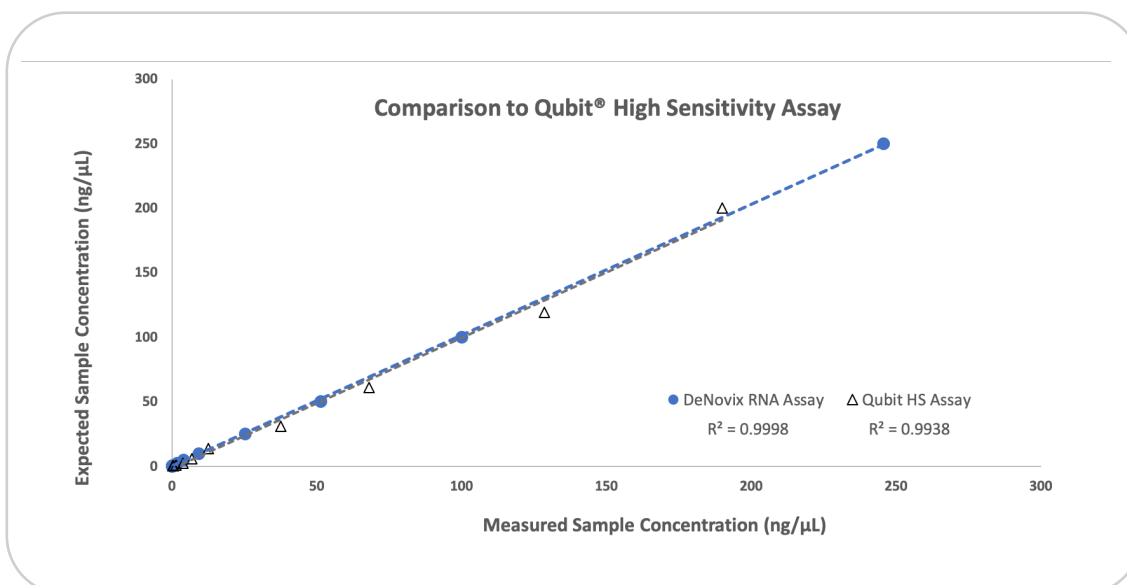


Figure 3. Comparison of Qubit® HS RNA and DeNovix RNA Assay

A comparison of the Qubit® HS RNA and DeNovix RNA Assay including linearity.



Performance Results (continued)

Table 1 lists the performance data that has been graphed in Figures 2 and 3. Five replicates were taken for each sample. The values in the expected category are based upon spectrophotometric analysis of each dilution to minimize the influence of pipetting errors.

Table 1: DeNovix RNA Assay performance data vs. Qubit® BR and HS Quantification Assays.

| RNA Assay Performance Data | | | | | | |
|----------------------------|------------------------------------|-------|------------------------------------|--------------|------------------------------------|--------------|
| Expected | DeNovix Assay measured on DS-11icx | | Qubit® BR Assay measured on Qubit® | | Qubit® HS Assay measured on Qubit® | |
| | ng/μL | %CV | ng/μL | %CV | ng/μL | %CV |
| 1500 | 1449.879 | 0.772 | Out of Range | Out of Range | Out of Range | Out of Range |
| 1000 | 990.358 | 0.426 | 1052 | 3.454 | Out of Range | Out of Range |
| 500 | 520.106 | 0.272 | 610.8 | 2.888 | Out of Range | Out of Range |
| 250 | 245.554 | 2.556 | 299.6 | 1.284 | Out of Range | Out of Range |
| 100 | 100.001 | 0.319 | 124.52 | 0.070 | 128.48 | 5.345 |
| 50 | 51.460 | 1.084 | 59.44 | 1.075 | 68.12 | 3.496 |
| 25 | 25.361 | 1.454 | 29.68 | 1.022 | 37.52 | 1.752 |
| 10 | 9.309 | 2.304 | 12.572 | 0.965 | 12.48 | 1.827 |
| 5 | 4.051 | 1.281 | 5.964 | 1.286 | 6.784 | 2.914 |
| 2.5 | 1.770 | 0.617 | 1.496 | 1.386 | 3.716 | 1.841 |
| 1 | 0.683 | 1.072 | 1.208 | 2.061 | 1.382 | 1.939 |
| 0.5 | 0.362 | 2.625 | 0.698 | 3.844 | 0.474 | 38.771 |
| 0.25 | 0.160 | 3.483 | Out of Range | Out of Range | 0.334 | 2.445 |
| 0 | 0 | 0 | Out of Range | Out of Range | Out of Range | Out of Range |

Summary

The DeNovix RNA Assay enables specific, highly sensitive RNA quantification across a wide dynamic range. Compared to Qubit®, the DeNovix RNA assay requires only a single kit to cover a wider concentration range with highly linear, reproducible data. Researchers benefit from a greater proportion of samples fitting within the assay limits, decreasing the requirement to dilute or concentrate samples and eliminating the time and expense of repeating assays.

DeNovix Fluorescence Assays

DeNovix offers a range of fluorescence quantification assays for DS-11 Series fluorometers and other compatible instruments. All assays offer sensitive and specific quantification for either dsDNA or RNA samples in the presence of contaminants.

| DeNovix Fluorescence Assay | Range |
|------------------------------|----------------------|
| dsDNA Broad Range | 0.1 – 2000 ng/μL |
| dsDNA High Sensitivity | 10 pg/μL – 250 ng/μL |
| dsDNA Ultra High Sensitivity | 0.5 – 300 pg/μL |
| RNA Assay | 0.25 - 1500 ng/μL |

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