Quantification of microRNA Using the Quantus™ Fluorometer and Qubit® microRNA Assay Kit

A Quantus™ Fluorometer Application Note

Materials Required:
- Qubit® microRNA Assay Kit (Thermo Fisher Scientific, Cat.# Q32880)
- 0.5mL PCR Tubes (Cat.# E4941)

Instrument Requirements:
- Quantus™ Fluorometer (Cat.# E6150)

Use the Quantus™ Fluorometer with Qubit® microRNA Assay Kits

Introduction

Micro RNA (miRNA) are small non-coding RNA molecules about 19-25 nucleotides in length that have been found in metazoans, plants, viruses, protists and slime mold, with more discoveries confirmed every day. Most current research focuses on either novel miRNA discovery or regulation of gene expression.

Regardless of the particular application, sample quantitation and subsequent normalization is necessary before adding sample to downstream analytical assays such as microarrays or next-generation sequencing. Few methods exist to selectively quantitate miRNA over other larger species that are co-purified in most RNA extraction procedures. One quantitation method is to use a modified RT-qPCR reaction. While this remains the most effective method for highly sensitive and accurate detection, some fluorescent dyes have been adapted to also selectively quantitate the smaller species in a much more rapid and simple format. The Qubit® microRNA Kit is one example of a fluorescent-based quantitation method that can give adequate miRNA estimation for many downstream assays.

This application note describes how to use the Qubit® microRNA Kit with the Quantus™ Fluorometer. The assay quantitates miRNA in solution at a final assay concentration of 0.05ng/µl-100ng/µl.

Methods

1. Prepare the Qubit® working solution by diluting the Qubit® microRNA reagent 1:200 in Qubit® microRNA buffer. Prepare enough working solution for total number of samples plus the required standards (2X). The standard tubes require 190µl of working solution and each sample tube requires anywhere from 180µl to 199µl.
2. Prepare standard tube by combining 190µl working solution and 10µl standard #2 (10ng/µl).
3. Prepare the blank tube by combining 190µl working solution and 10µl standard #1 (0ng/µl).

4. Prepare sample tubes by combining 1–20µl sample and working solution to a final of 200µl.
5. Vortex all tubes for 2–3 seconds to mix.
6. Allow all tubes to incubate at room temperature for 2 minutes.
7. Create a user-defined protocol on the Quantus™ Fluorometer
   a. From the home screen, select the "Protocols" tab. Then scroll down and select "User defined empty" and rename the protocol.
   b. Set the standard value to 0.5. Select the "Blue" fluorescence channel.
   c. Select "Save".
8. Select the newly created protocol and select the correct units and sample volume.
9. To calibrate the protocol, select "Calibrate". Place the blank tube into the holder, close the lid, and select "Read Blank". Repeat for the standard sample, this time selecting "Read Std". Save the calibration data by selecting "Save".
10. To quantitate samples, place sample tube into the tube holder, and close the lid. The instrument will automatically measure fluorescence when the lid is closed, and the calculated nucleic acid concentration will be displayed.

Results

Table 1: Concentration Values (ng/µl) of Serially Diluted Standard as Determined by the Quantus™ and the Qubit® 3.0 Fluorometers (n = 3).

Expected Concentration (ng/µl)	Quantus™ Fluorometer		Qubit® 3.0 Fluorometer	
	Average	Standard Deviation	Average	Standard Deviation
10	9.767	0.208	9.660	0.171
5	4.933	0.058	4.967	0.070
2.5	2.433	0.021	2.480	0.020
1.25	1.220	0.010	1.257	0.006
0.625	0.609	0.004	0.635	0.001
0.313	0.309	0.004	0.323	0.003
0.156	0.157	0.006	0.169	0.004

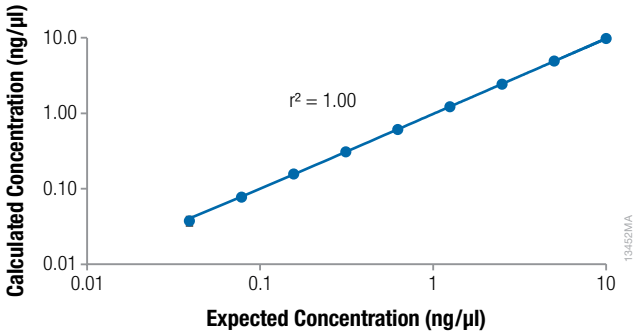

Figure 1. miRNA quantitation data generated using the Quantus™ Fluorometer and Qubit® microRNA Kit. The 10ng/µl standard from the Qubit® microRNA Assay Kit was twofold serially diluted (10ng/µl to 0.039ng/µl) in Qubit® microRNA buffer. Ten microliters of each sample was combined with 190µl of Qubit® working solution. Concentration values (ng/µl) of serially diluted standard determined by the Quantus™ Fluorometer (n = 3) were plotted against expected values.

Conclusions

The Quantus™ Fluorometer, in combination with the Qubit® microRNA Assay Kit, can measure low levels of miRNA. Results demonstrate concordance with the expected values (Figure 1). Measurements taken using both the Quantus™ Fluorometer and Qubit® 3.0 instruments result in nearly identical concentration values (Table 1).

Ordering Information

Product	Cat.#
Quantus™ Fluorometer	E6150