Measuring RNA Concentration Using the Quantus™ Fluorometer with the Qubit® RNA Assay Kit

Promega Corporation

Materials Required

- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Axygen Cat.# PCR-05-C, available through Fisher or VWR)
- Qubit® RNA Assay Kit (Life Technologies Cat.# Q32852)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocol: *Quantus™ Fluorometer Operating Manual* #TM396 is available at: **www.promega.com/protocols/**

Detecting and quantitating small amounts of RNA are important steps in many molecular biology techniques. These include measuring yields of in vitro transcribed RNA and measuring RNA concentration before performing Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library construction, reverse transcription PCR and differential display PCR.

The most commonly used technique to determine nucleic acid concentration is measuring absorbance at 260nm (A_{260}). The major disadvantages of the absorbance-based method include: the inability to distinguish among DNA (both single- and double-stranded), RNA and nucleotides, interference caused by contaminants commonly found in nucleic acid preparations, and the relative insensitivity of the assay (traditional spectrophotometric assays cannot determine nucleic acid concentrations below 2µg/ml). The use of sensitive, fluorescent nucleic acid stains alleviates many of these problems.

The Qubit® RNA Assay Kit can be used with the Quantus™ Fluorometer. The assay provides an accurate and selective method to quantify high-abundance RNA samples without quantitating DNA, protein or free nucleotides. Common contaminants, such as salts, free nucleotides, solvents, detergents or protein, are well tolerated in the assay. The assay kit is designed to quantitate 5–100ng of RNA in a 200µl assay.

This Application Note describes the protocol for using the Qubit® RNA Assay Kit with the Quantus™ Fluorometer.

Protocol

1. Create a custom protocol on the Quantus™ Fluorometer by selecting "New" from the menu list on the Protocol screen, and name the protocol by using the up or down buttons. Enter the standard value of 0.5ng/µl. Select the Red channel, and save the protocol.

   Note: The standard value was calculated by dividing the RNA amount of Standard #2, which is supplied with the Qubit® RNA Assay Kit, (100ng) by the assay volume (200µl).

2. Equilibrate all reagents to room temperature.

3. Prepare Qubit® working solution by diluting the Qubit® RNA reagent 1:200 in Qubit® RNA buffer. For example, add 10µl of Qubit® RNA reagent to 1,990µl of Qubit® RNA buffer, and mix. Prepare 200µl of Qubit® working solution for each standard and unknown sample.
4. Prepare the two standard samples by adding 10µl of each standard to 190µl of Qubit® working solution.

   Note: The Qubit® RNA Assay Kit provides standards labeled as #1 and #2. Standard #1 is a blank solution, and Standard #2 contains 10ng/µl RNA.
5. Prepare the unknown sample by combining 1–20µl of sample with enough Qubit® working solution to bring the final assay volume to 200µl.
6. Vortex tubes for 2–3 seconds, and incubate at room temperature for 2 minutes, protected from light.
7. Select the custom protocol created in Step 1. Go to the Calibration screen and read the two prepared standards. Standard #1 is the blank sample, and Standard #2 is the standard sample. Save the calibration.
8. Enter the volume of the unknown sample and desired concentration units.

   Note: This volume is the amount of sample that is added for the quantitation. For example, if 1µl of sample was mixed with 199µl of reagent working solution for a total volume of 200µl in the tube, then the volume entered on this screen is 1µl.
9. Place the unknown sample 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.

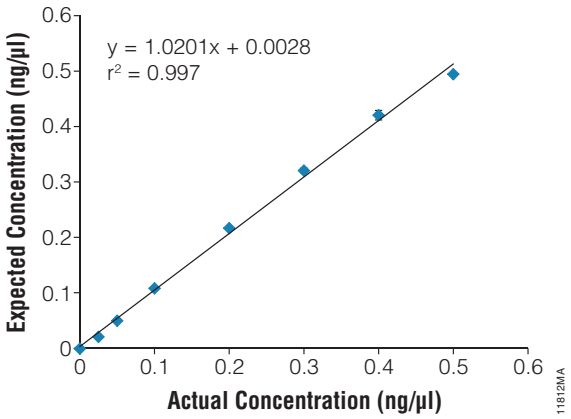

Figure 1. Measuring RNA concentration using the Qubit® RNA Assay Kit and the Quantus™ Fluorometer. Standard curve was generated per manufacturer's instructions to demonstrate the linearity of the Quantus™ Fluorometer. Samples were run in duplicate.

Quantus is a trademark of Promega Corporation. Qubit is a registered trademark of Life Technologies Corporation

Products may be covered by pending or issued patents or may have certain limitations. Please visit our web site for more information.