Measuring Double-Stranded DNA Concentration Using the Quantus™ Fluorometer with the Qubit® dsDNA BR 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® dsDNA BR Assay Kit (Life Technologies Cat.# Q32850)

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 DNA are important steps in a wide variety of biological applications. These include standard molecular biology techniques such as constructing cDNA libraries, purifying DNA fragments or subcloning, quantitating DNA amplification products and detecting DNA molecules in drug preparations.

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® dsDNA BR (Broad Range) Assay Kit can be used with the Quantus™ Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA and designed to quantitate 2–1,000ng of DNA in a 200µl assay.

This Application Note describes the protocol for using the Qubit® dsDNA BR 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 5ng/µl. Select the Blue channel, and save the protocol.

   Note: The standard value was calculated by dividing the DNA amount of Standard #2, which is supplied with the Qubit® BR Assay Kit, used (1,000ng) by the assay volume (200µl).

2. Equilibrate all reagents to room temperature.

3. Prepare Qubit® working solution by diluting the Qubit® dsDNA BR reagent 1:200 in Qubit® dsDNA BR buffer. For example, add 10µl of Qubit® dsDNA BR reagent to 1,990µl of Qubit® dsDNA BR 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® dsDNA BR Assay Kit provides standards labeled as #1 and #2. Standard #1 is a blank solution, and standard #2 contains 100ng/µl dsDNA.
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.

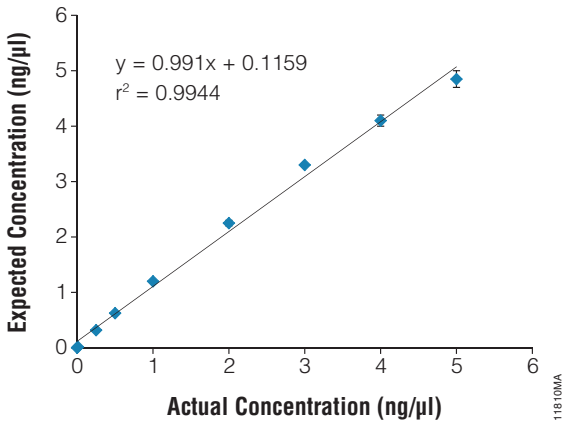

Figure 1. Measuring dsDNA concentration using the Qubit® dsDNA BR Assay Kit and Quantus™ Fluorometer. Standard curve was generated per manufacturer's instructions to demonstrate the linearity of the Quantus™ Fluorometer. Samples were run in duplicate.