Measuring Fluorescein using the Quantus™ Fluorometer

Promega Corporation

Materials Required

- Fluorescein (e.g., Sigma Cat.# 46960-100G-F)
- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Axygen Cat.# PCR-05-C, available through Fisher or VWR)

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

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

Fluorescein is a synthetic organic compound with wide-ranging biological application as a label for antibodies and other probes used in fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as Western blotting and ELISA.

Fluorescein can be measured using the Quantus™ Fluorometer (Cat.# E6150) as a quantitation step in a variety of applications. Because fluorescein is autofluorescent, no dye chemistries are necessary for quantitation on the Quantus™ Fluorometer. The only components required are a standard of known concentration and a blank. Fluorescein has been tested on the Quantus™ Fluorometer in the range of 830pg/µl–13fg/µl (2500nM–38pM) in a 200µl assay.

This Application Note describes the protocol for measuring fluorescein using the Quantus™ Fluorometer.

Protocol

1. Create a custom protocol on the Quantus™ Fluorometer by selecting "New" from the menu on the Protocol screen, and name the protocol using the up or down buttons. Enter the standard value of 830pg/µl. Select the Blue channel, and save the protocol.

   Note: The standard value was calculated by dividing the amount of fluorescein added to the standard (166ng) by the assay volume (200µl).

2. Equilibrate all reagents to room temperature.
3. Prepare the Fluorescein Standard by diluting a fluorescein stock to 830pg/µl in water. The Blank sample is 200µl of water.
4. Prepare the unknown samples by combining desired amount of sample with enough water (or preferred diluent) to bring the final assay volume to 200µl.
5. Select the custom protocol created in Step 1. Go to the Calibration screen and read the Fluorescein Standard and Blank samples prepared in Step 3. Save the calibration.

6. Enter the volume of the unknown samples and the desired concentration units.

   Note: This volume is the amount of sample that is added for the quantitation. For example, if 1µl of sample was diluted to the 200µl assay volume, then the volume entered on this screen should be 1µl.

7. 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 concentration will be displayed.

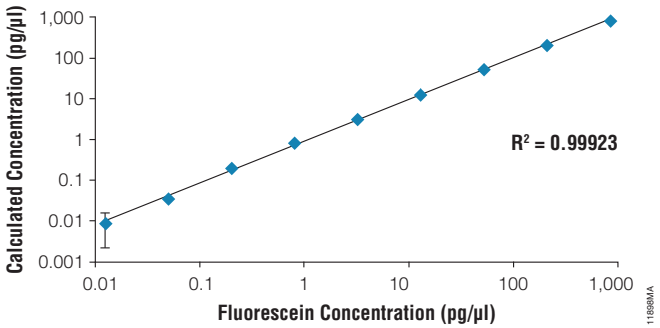

Figure 1. Measuring Fluorescein concentration using the Quantus™ Fluorometer. The standard curve was generated from a dilution series of fluorescein dye to demonstrate linearity of the data generated using the Quantus™ Fluorometer. Samples were run in duplicate.

