

# Measuring GFP using the Quantus™ Fluorometer

Promega Corporation



## Materials Required

- GFP Quantification Kit (BioVision, Inc., Cat.# K815-100)
- 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/](http://www.promega.com/protocols/)

Green Fluorescent Protein (GFP) is a small, stable and versatile protein that, because of its hexapeptide chromophore, displays unique autofluorescence with no needed co-factors. Today, GFP has many applications in almost all organisms and all major cellular compartments. It has been used in cellular biology as a cell marker, fusion tag, and reporter gene. GFP has also been used as an active indicator for protease action, transcription factor dimerization, calcium sensitivity, and to quantitatively monitor gene expression.

GFP can be measured with the Quantus™ Fluorometer (Cat.# E6150) as a quantitation step in a variety of applications. Because GFP is autofluorescent, no dye chemistries are necessary for quantitation. The only required components are a standard of known GFP concentration and a blank. GFP has been tested on the Quantus™ Fluorometer down to 80ng/ml in a 200µl assay. This testing was performed using the GFP Standard provided in the GFP Quantification Kit produced by BioVision, Inc. (Cat.# K815-100).

This Application Note describes the protocol for measuring Green Fluorescent Protein 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 by using the up or down buttons. Enter a standard value of 4000ng/ml. Select the Blue channel, and save the protocol.

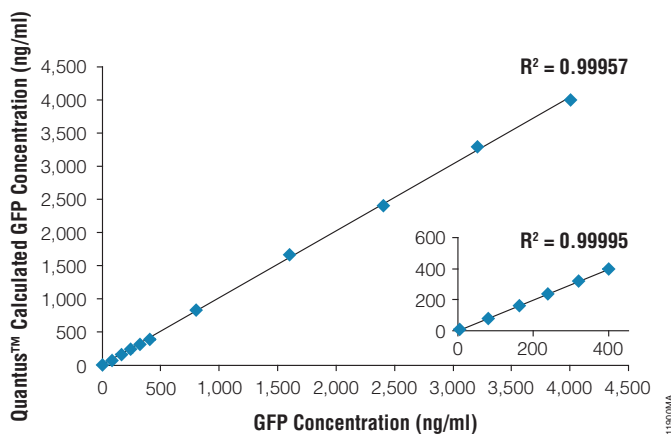
**Note:** The standard value was calculated by dividing the amount of GFP added to the standard (800ng) by the assay volume (200µl).

2. Equilibrate all reagents to room temperature.
3. Prepare the GFP Standard by resuspending 100µg GFP with 100µl GFP Assay Buffer (provided in the GFP Quantification Kit). Dilute 100-fold to create a 10ng/µl GFP solution. Add 80µl of this GFP solution to 120µl of Assay Buffer in a 0.5ml PCR tube. The Blank is 200µl of GFP Assay Buffer.
4. Prepare the unknown samples by combining the desired amount of sample with enough GFP Assay Buffer to bring the final volume to 200µl.
5. Select the custom protocol created in Step 1. Go to the Calibration screen and read the 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 added to 199µl GFP Assay Buffer, 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 GFP concentration using the Quantus™ Fluorometer.** The standard curve was generated using the GFP Quantitation Kit protocol, scaling volumes up to 200µl, to demonstrate the linearity of the Quantus™ Fluorometer. Samples were run in duplicate.

Quantus is a trademark of Promega Corporation.

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

