A Modulus™ Single Tube Fluorometer Method for

Quantitation of Living Color® rAcGFP1 in Gene Expression and Protein Visualization Studies

1. INTRODUCTION

Clontech Living Colors™ green fluorescent protein from *Aequorea coerulescens* (AcGFP1) is a new alternative to monomeric enhanced GFP (EGFP). The spectral properties of AcGFP1 closely resembles those of EGFP, with AcGFP1 having an excitation maximum of 475 nm and an emission maximum of 505 nm (compared to 484/510 nm of EGFP). In addition, the AcGFP1 protein has 94% homology to EGFP at the amino acid level and is very stable, allowing the examination of fluorescence over an extended period of time. AcGFP1 can be used as a fluorescent marker for gene expression in a variety of organisms from bacteria to higher plants and animals. Additionally, AcGFP1 can be used to visually monitor the real-time movement of your protein of interest.

Turner BioSystems' Modulus™ Single Tube Fluorometer can detect as low as 5 pg/? L and up to 10 ng/µL of recombinant AcGFP1 (rAcGFP1).

2. MATERIALS REQUIRED

From Turner BioSystems:

- Modulus™ Single Tube Fluorometer (P/N 9200-001 or 9200-002)
- Fluorescence Optical Kit–BLUE (P/N 9200-040)
- Minicell Adaptor Kit (P/N 9200-928)

From BD Biosciences Clontech:

- Affinity purified recombinant *Aequorea coerulescens* green fluorescent protein (rAcGFP1). (Catalog No. 632502)

Other Materials:

- Adjustable p200 Volume Pipettor and Tips
- Adjustable p20 Volume Pipettor and Tips
- TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 1.5 mL microfuge tubes
- Test tube rack
- Nitrile, vinyl, or latex gloves

Storage Conditions: Store rAcGFP1 at –20°C.

3. INSTRUMENT SET-UP

3.1 Power OFF the Modulus. Install the Fluorescence Optical Kit-BLUE module into the sample compartment.

3.2 Turn ON the Modulus, confirm that you are using the Blue optical kit and allow the instrument to warm up for 5 minutes.

3.3 Insert minicell adaptor into the optical kit with the tab facing farthest away from you.

4. PREPARING STANDARD CURVE

Prepare dilutions of rAcGFP1 according to the table below:

Dilution #	ml of Stock rAcGFP1 (1ng/ml)	ml of Stock rAcGFP1 (100ng/ml)	ml of TE Buffer	Final concentration (ng/ml)
1	-	-	100	0
2	1	-	99	0.01
3	5	-	95	0.05
4	10	-	90	0.1
5	25	-	75	0.25
6	50	-	50	0.5
7	100	-	0	1
8	-	2.5	97.5	2.5
9	-	5	95	5
10	-	10	90	10

***Table 1. Preparation of rAcGFP1 dilutions**. To make a range of rAcGFP1 dilutions, add the appropriate volumes of stock rAcGFP1 and TE Buffer as shown in the table.*

4.1 Add 100 µL of each rAcGFP1 dilution into a separate minicell cuvette. **NOTE:** Make sure there are no bubbles in the cuvette when transferring solutions. Bubbles will cause erratic readings.

4.2 Insert minicell cuvette containing sample into the minicell adaptor. Touch "Measure Fluorescence Raw."

4.4 Record the value and repeat steps 4.2-4.3 for your remaining samples.

4.5 Plot the FSU values versus the concentration of your dilutions to obtain a standard curve.

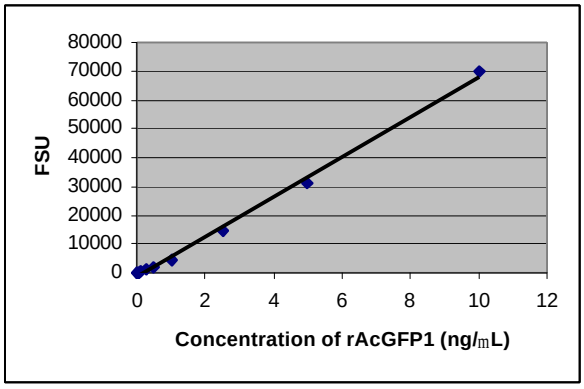

***Figure 1. Concentration of rAcGFP1 in ng/mL vs. FSU**. Various dilutions of rAcGFP1 plotted against FSU values. The Modulus is able to detect as low as 5 pg/mL and up to 10 ng/mL of rAcGFP1.*

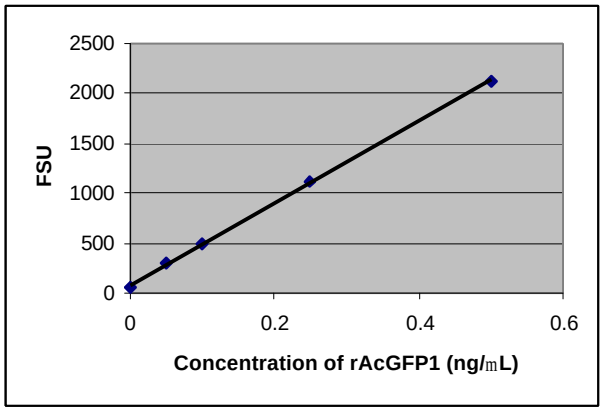

***Figure 2. Concentration of rAcGFP1 in ng/mL vs. FSU**. Close up version of Figure 1 from 0 to 0.5 ng/mL*

4.6 Use the standard curve to determine the concentration of each of your unknown samples.

5. CALIBRATION

5.1 Alternatively, you may calibrate the Modulus with your rAcGFP1 dilutions before reading unknown samples. To do so, press "Calibrate."

5.2 Calibrate the Modulus with as many as 5 of the dilutions prepared in Table 1. Choose "ng/µL" for the unit of measure. Use the 0 ng/µL standard for the blank solution. To optimize performance and accuracy, choose the 5 dilutions that are closest in range to a typical sample. Enter the standards in order of increasing concentration.

5.3 Save the calibration for future use (optional).

5.4 Insert unknown sample into the Modulus and press “Measure Fluorescence.” **NOTE:** It is not necessary to run a standard curve after calibration. All subsequent readings will report in ng/μL final concentration.

5.5 The final concentration of the sample appears on the touchscreen.

6. ABOUT CLONTECH LABORATORIES

Living Colors® rAcGFP1 Fluorescent Protein is from Clontech Laboratories.

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7. ABOUT TURNER BIOSYSTEMS, INC.

Modulus is a trademark of Turner BioSystems, Inc. Orders for Turner BioSystems’ products may be placed by:

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