A Modulus™ Microplate Fluorometer Method for

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

INTRODUCTION

Clontech Living Colors® recombinant green fluorescent protein from *Aequorea coerulescens* (rAcGFP1) is a new alternative to monomeric enhanced GFP (EGFP). The spectral properties of rAcGFP1 closely resemble those of EGFP; rAcGFP1, excitation maximum is 475 nm and emission maximum is 505 nm as compared to 484/510 nm for EGFP. In addition, the rAcGFP1 protein has 94% homology to EGFP at the amino acid level and is very stable, allowing flurorescence examination over an extended period of time. rAcGFP1 can be used as a fluorescent marker for gene expression reporter assays in organisms varying from bacteria to higher plants and animals.

This application note describes a method successfully used to quantitate rAcGFP1 using the Turner BioSystems Modulus™ Microplate Fluorometer. This instrument can detect concentrations of rAcGFP1 ranging from as low as 10 pg/µL and up to 10 ng/µL in 96-well microplate format.

MATERIALS REQUIRED

From Turner BioSystems:

- Modulus™ Microplate Multimode Reader
- Fluorescence Optical Kit – Blue, 460/515 - 580 nm
- 96-well, black microplate (Greiner Bio, FluoTrac 200, 655076)

From Clontech:

- Affinity-purified recombinant *Aequorea coerulescens* green fluorescent protein (rAcGFP1, Catalog No. 632502).
  Note: rAcGFP1 should be stored at –20°C.

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

EXPERIMENTAL PROTOCOL

1. Instrument Set Up

- Insert the Blue Fluorescence Optical Kit (excitation 460 nm, emission 515 - 580 nm) into the Modulus™ Microplate according to the Operating Manual.
- From the Home screen, touch Select Protocol and follow the wizard. Select preset protocol for rAcGFP1. Select fluorescence and the Preset tab for rAcGFP1; Finish.
- The Instrument Control screen shows the reading parameters: 1 sec. integration, Blue Optical Kit, and all plate wells are selected.
  Note: If necessary, use the Plate/Well selection icon to select or deselect wells to read. Green is selected and gray is deselected.
- Refer to the on-screen Help topics, Quick Start Guide, or Operating Manual for detailed instructions.

2. Preparing Standard Curve

- Prepare dilutions of rAcGFP1 according to the table below.

Sample #	µL of Stock rAcGFP1 (1 ng/µL)	µL of Stock rAcGFP1 (100 ng/µL)	µL of TE Buffer	Final Conc. (ng/µL)
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 appropriate volumes of stock rAcGFP1 and TE Buffer as shown in the table.

- Add 200 µL of sample to each well. **Note:** If necessary, use the Plate/Well selection icon to select or deselect wells to read. Green is selected and gray is deselected.
- Open the instrument door by using the Door icon on the touch screen. Place well A1 at the top right corner of the microplate sample tray. Close the door by using the Door icon.
- Select the Start icon on the touch screen to begin reading.
- The Results screen displays RFU values immediately after each well is measured.
- Once all measurements are complete, data can be transferred to an external computer for further data analysis in Excel by using the provided USB flash drive.
- Remove the plate after measurement.
- Plot RFU values versus dilution concentrations to obtain a standard curve.

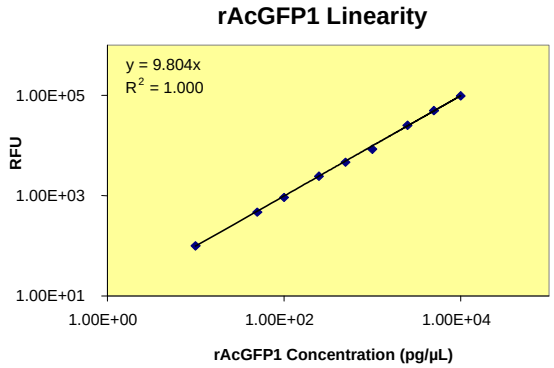

Figure 1. Concentration of rAcGFP1 in pg/µLversus RFU. Various dilutions of rAcGFP1 plotted against RFU values. The Modulus™ Microplate Fluorometer is able to detect as low as 10 pg/µL and up to 10 ng/µL of rAcGFP1.

- Use the standard curve to determine each samples' unknown concentration.

RESULTS

Sensitivity: < 10 pg/µL

Dynamic Range: Dynamic range of up to four orders of magnitude

Minimum Detection Limit: 0.28 pg/µL (as calculated using three times standard deviation of the assay background)

CONCLUSION

The Modulus™ Microplate Fluorometer offers superior sensitivity and dynamic range for detection of Clontech Living Colors® rAcGFP1, enhancing the results of gene expression and real-time protein visualization studies. The Modulus™ Microplate Fluorometer achieves its superior performance with a dedicated fluorescence detector instead of sharing the detector with other detection modes. The individual optical kit with solid-state optics and a powerful wavelength-matched LED delivers excellent sensitivity and dynamic range.

The modular approach of the Modulus™ Microplate Fluorometer allows for instrument capability expansion as needs in the lab change. Luminescence and/or absorbance detection modules as well as other accessories can be added after the initial purchase.

The superior performance, ease of use, and utmost flexibility of the Modulus™ Microplate make it an ideal microplate reader for today's life science laboratory.

TRADEMARKS

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