

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

Sherry Wang, Ph.D.

Turner BioSystems, Inc.

*This report describes how to quantify amounts of rAcGFP1, Clontech's new Living Colors® recombinant green fluorescent protein from *Aequorea coerulea*, using a Turner BioSystems Modulus™ Fluorometer. Concentrations of rAcGFP1 ranging from as low as 5 picograms/μl up to 10 nanograms/μl can be determined using this instrument. The new rAcGFP1 protein may be used as a fluorescent marker for gene expression in a variety of organisms, ranging from bacteria to higher plants and animals. It can also be used to visually monitor the real-time movement of your protein of interest. rAcGFP1 is one of two new 6xHN-tagged, purified, recombinant fluorescent proteins (FPs) offered by Clontech. The other is rDsRed-Monomer. The spectral properties of these recombinant FPs are identical to those of the native proteins expressed in mammalian cells, so the recombinant FPs may be used to standardize fluorometric measurements. They can also be used as standards in polyacrylamide gels and in Western blots, using the appropriate antibodies. Both recombinant FPs are known to be well-tolerated in mammalian cells, making them suitable for microinjection studies.*

The Clontech Living Colors® recombinant green fluorescent protein from *Aequorea coerulea* (rAcGFP1) is a new alternative to monomeric enhanced GFP (EGFP) (1, 2). The spectral properties of AcGFP1 closely resemble those of EGFP, with rAcGFP1 having an excitation maximum of 475 nm and an emission maximum of 505 nm (compared to corresponding maxima of 484 nm and 510 nm, respectively, for EGFP). In addition, the rAcGFP1 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.

Below, we describe a method that has been used successfully to quantify rAcGFP1 using the Turner BioSystems Modulus™ Fluorometer. This instrument

can detect concentrations of rAcGFP1 ranging from 5 pg/μl to 10 ng/μl. Data demonstrating this range of detection is provided. The Modulus is a multi-functional single-tube instrument that features an easy-to-use color touchscreen and provides significant flexibility for measuring fluorescence, luminescence, and absorbance. We indicate the materials required and describe the instrument setup, standard curve creation, and calibration approach that allow accurate and sensitive quantification of rAcGFP1.

Materials required

The following materials are required to perform the quantification:

From Clontech:

- Affinity-purified recombinant *Aequorea coerulea* green fluorescent protein (rAcGFP1) (Cat. No. 632502)

From Turner BioSystems:

- Modulus Fluorometer (Cat. No. 9200-001 or 9200-002)
- Fluorescence Optical Kit-BLUE (Cat. No. 9200-040)
- Minicell Adaptor Kit (Cat. No. 9200-928)

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

Note that rAcGFP1 should be stored at -20°C.

Instrument setup

Follow the instructions below to prepare the Modulus Fluorometer for operation.

1. Power OFF the Modulus. Install the Fluorescence Optical Kit-BLUE module into the sample compartment.
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. Insert the minicell adaptor into the optical kit with the tab facing farthest away from you.

Table I: Preparation of rAcGFP1 Dilutions

Dilution	μl of Stock rAcGFP1 (1 ng/ml)	μl of Stock rAcGFP1 (100 ng/ml)	μl 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

Quantification of Living Colors® rAcGFP1 in Gene Expression and Protein Visualization Studies...continued

Preparing a standard curve

Follow the instructions below to prepare the standard curve that you can use to determine the rAcGFP1 concentrations of your unknown samples (Figures 1 and 2).

1. Prepare dilutions of rAcGFP1 using the volumes shown in Table I. To make a range of rAcGFP1 dilutions, add the appropriate volumes of stock rAcGFP1 and TE Buffer as shown in the table.
2. 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.
3. Insert minicell cuvette containing sample into the minicell adaptor. Touch “Measure Fluorescence Raw.”
4. Record the value and repeat steps 2 and 3 for your remaining samples.
5. Plot the FSU values versus the concentration of your dilutions to obtain a standard curve.

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

Calibration

1. As an alternative to using the standard curve, you may calibrate the Modulus with your rAcGFP1 dilutions before reading unknown samples. To do so, first press “Calibrate.”
2. Calibrate the Modulus with as many as five of the dilutions prepared in Table I. 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 five dilutions that are closest in range to a typical sample. Enter the standards in order of increasing concentration.
3. Save the calibration for future use (optional).

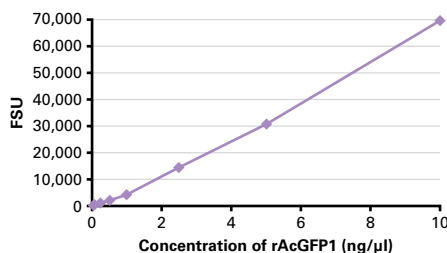


Figure 1. Standard curve. Concentration of rAcGFP1 in ng/µl vs. FSU. Various dilutions of rAcGFP1 are plotted against FSU values. The Modulus is able to detect rAcGFP1 concentrations as low as 5 pg/µl and up to 10 ng/µl.

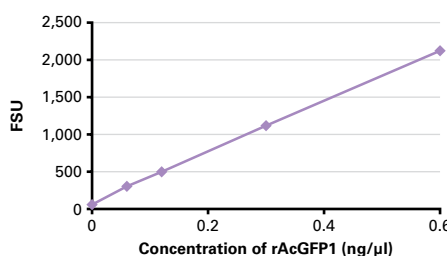


Figure 2. Standard curve (closeup). Concentration of rAcGFP1 in ng/µl vs. FSU. Close-up version of Figure 1 from 0 to 0.6 ng/µl.

4. Insert an unknown sample into the Modulus and press “Measure Fluorescence.”
5. The final concentration of the sample appears on the touchscreen of the Modulus.

NOTE: It is not necessary to run a standard curve after calibration. All subsequent readings will be reported in ng/µl final concentrations.

Use of Clontech’s new Living Colors rAcGFP1 protein with the Turner BioSystems Modulus Fluorometer will allow you to greatly enhance the results of your gene expression and real-time protein visualization studies.

References

1. Living Colors Fluorescent Protein Vectors (July 2006) *Clontechiques XXI*(1):16–17.
2. Living Colors Fluorescent Protein Vectors (October 2005) *Clontechiques XX*(2):18–20.

Product	Size	Cat. No.	Price
rAcGFP1 Protein	100 µg	632502	\$265.00
rDsRed-Monomer Protein	100 µg	632503	\$265.00
rGFP Protein	100 µg	632373	\$304.00
rDsRed2 Protein	100 µg	632436	\$273.00
rDsRed-Express Protein	100 µg	632437	\$273.00
rGFPuv Protein	100 µg	632369	\$279.00
rHcRed1 Protein	100 µg	632438	\$273.00

Prices are subject to change without notice.

Related Products

- Living Colors® A.v. Monoclonal Antibody (JL-8) (Cat. Nos. 632380 & 632381)
- Living Colors® Full-Length A.v. Polyclonal Antibody (Cat. Nos. 632459 & 632460)
- Living Colors® A.v. Peptide Antibody (Cat. Nos. 632376 & 632377)
- Living Colors® GFP Monoclonal Antibody (Cat. No. 632375)
- Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496)
- Living Colors® Fluorescent Protein Vectors (many)

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale. Clontech, Clontech logo, and all other trademarks are the property of Clontech Laboratories, Inc. Clontech is a Takara Bio Company. Copyright 2006.

Notice to Purchaser

For CMV Sequence

The CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 assigned to the University of Iowa Research Foundation.

For DsRed-Express

Patent Pending.

DsRed-Monomer

This product is the subject of pending U.S. patent applications.

For Living Colors® Products

AcGFP1, DsRed, HcRed, AsRed, AmCyan, ZsGreen, ZsYellow, and their variants:

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.