Measuring Intracellular Enhanced Green Fluorescent Protein with the VersaFluor™ Fluorometer

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Introduction

The Green Fluorescent Protein (GFP) derived from Aqueorius victoria has recently become a popular choice as a reporter gene. GFP can be used, for example, to study promoter function and to track the expression, localization, and function of proteins to which it has been recombinantly fused.[1,2] When stimulated by light the molecule fluoresces without addition of any cofactors or substrates. For our studies we have used the Enhanced Green Fluorescent Protein gene (EGFP), a red-shifted variant of the wild-type protein, as a reporter gene in Jurkat cells. EGFP has an excitation maximum at 488 nm, and emission maximum at 507 nm. It is very resistant to photobleaching and is reported to maintain stable fluorescence from pH 7.0 to 11.5.[3] In fluorometric assays, purified EGFP can be measured at concentrations in the low nanogram range.

In recent experiments we have shown that intracellular EGFP can be assayed using a VersaFluor fluorometer. Using the EX490/10 and EM510/10 filter set, we compared pEGFP-n2 transfected Jurkat cells with wild-type Jurkat cells. To approximate the amount of EGFP produced by transfected Jurkat cells, a standard curve with recombinant Enhanced Green Fluorescent Protein (rEGFP) was also generated. The methods and results from those experiments are reported here.

Methods

Jurkat cells (clone E6-1 from ATCC), a leukemic human T-cell line, were maintained in RPMI 1640 media with 2 mM L-glutamine and supplemented with 2.5 g/l glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, in addition to 10% fetal bovine serum.

The enhanced green fluorescent protein vector pEGFP-n2 (Clontech Laboratories, Inc.) was transfected into Jurkat cells using a Gene Pulser® II system with the Capacitance Extender Plus. The electroporation was conducted with a 0.4 cm cuvette with 310 volts and 950 µF using 10 µg of linearized pEGFP-n2 vector.[4] Forty-eight hours post-transfection Jurkats were selected for neomycin resistance using G418 at a dose of 1 mg/ml. After 2 weeks, G418 was reduced to a concentration of 0.5 mg/ml.

Both untransfected and pEGFP-n2 transfected cells in exponential growth were harvested and washed twice with PBS (Phosphate Buffered Saline) and then suspended in PBS buffer. Viable cells were then counted by trypan blue exclusion using a hemacytometer and diluted to a concentration of 1.0 x 107 cells/ml. Cells from both groups were then diluted into 500 µl aliquots at concentrations of 1.0 x 105, 3.16 x 105, 1.0 x 106, and 3.16 x 106 cells/ml. These values correspond to log con-centrations of 0.0, 0.5, 1.0, and 1.5 x 100,000 cells/ml, respectively.

Fluorescence was then measured using a VersaFluor fluorometer. An EX490/10 excitation filter was used in conjunction with an EM510/10 emission filter. The gain was set to “medium” and 250 µl disposable microcuvettes were used. The instrument was zeroed with a 200 µl sample of PBS buffer. After equilibrating at zero, the microcuvette was removed and a 200 µl sample of 1.0 x 10^5 untransfected cells/ml was analyzed in a new microcuvette. After equilibrating for approximately 10 seconds, the relative fluorescent units (RFUs) were recorded and then the microcuvette was discarded. Then a 200 µl sample of 3.16 x 10^5 untransfected cells/ml was analyzed. This procedure continued until each dilution of cells from both the pEGFP-n2 and wild-type Jurkat groups were analyzed once. Then, the fluorometer was re-zeroed, and duplicate measurements were taken for each dilution from both cell groups.

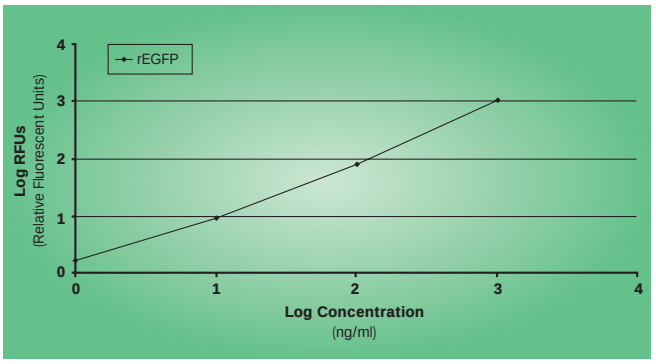

Fig. 1. Standard curve of purified rEGFP suspended with 1 x 10^6 wild type Jurkat cells/ml in PBS. This curve reflects the log values calculated from RFUs generated by the VersaFluor fluorometer with the EX490/10 and EM510/10 filters. All points are the mean of triplicate measurements ± S.D.

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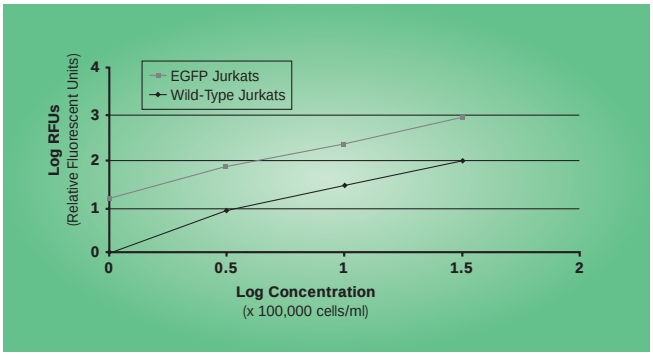

Fig.2 Comparison of fluorescence intensity of wild-type vs pEGFP transfected Jurkat cells. These curves reflect the log values calculated from RFUs generated by the VersaFluor fluorometer with the EX490/10 and EM510/10 filters. All points are the mean of duplicate measurements ± S.D.

A standard curve using purified recombinant EGFP was then generated. To approximate the intracellular EGFP measured with the pEGFP-n2 transfected Jurkats, rEGFP (Clontech Laboratories, Inc.) was diluted into 1.0 x 10^5 wild-type Jurkat cells/ml at concentrations of 0, 1, 10, 100, and 1,000 ng/ml. Using the settings described above for the VersaFluor fluorometer, fluorescence was measured. A 200 µl sample of wild-type Jurkat cells without rEGFP was zeroed and established as baseline. Then a sample with 1 ng/ml rEGFP was analyzed, followed by the 10, 100, and 1,000 ng/ml samples. This procedure was then repeated twice more, so that triplicate values were obtained for each concentration of rEGFP.

Results and Discussion

A standard curve with purified rEGFP was generated as shown in Figure 1. Although soluble EGFP is not a perfect quantitative standard for intracellular EGFP measurement, an estimation of intracellular EGFP can be made.

As shown in Figure 2, pEGFP-n2 transfected Jurkat cells exhibited considerably more fluorescence in the 505–515 nm range than did wild-type Jurkat cells. Comparison of fluorescence on a log scale shows that both cell groups generated near linear intensity curves, with a difference in net fluorescence of approximately one log order.

If the net EGFP fluorescence is compared with the rEGFP standard curve in Figure 1, it is evident that the net fluorescence of pEGFP-n2 transfected Jurkats equates to approximately 10ng/ml rEGFP.

In conclusion, this experiment shows that the VersaFluor fluorometer can be used to assay for the presence of intracellular EGFP in intact, viable Jurkat cells.

References

1 *Kain, S. R., Adams, M., Kondepudi A., Yang T. T., Ward, W. W., and Kitts, P.,* Biotechniques*, 19, 650-655 (1995).*
2 *Ogawa, H., Inouye S., Tsuji, F. I., Yasuda, K., and Umesono K.,* PNAS*, 92, 11899-11903 (1995).*
3 *Ward, W. W., personal communication*
4 *Barry, P., Pratte-Lowe, E., and Luciw, P.,* Bio-Rad Bulletin 1349

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