

Benchmarks

Measurement of Human Growth Hormone Using a Chemiluminescence Assay and a "Glow" Luminometer

BioTechniques 20:48-50 (January 1996)

The gene that codes for human growth hormone (hGH) is used as a reporter gene to assess changes an effector may have on gene expression of a specific DNA sequence (6). hGH is secreted into the medium of transfected cells following the addition of an appropriate effector; measurement of hGH levels allows for quantification of the degree of activation (or suppression) of gene expression relative to untreated transfected cells.

Many laboratories use the gene that codes for firefly luciferase as a reporter (1,5) because it affords extreme sensitivity in monitoring changes in gene expression. While luciferase activity can be monitored using a "flash" luminometer (one that quantifies a pulse of light), other types of luminometers ("glow" or "integrating") allow for integration of light produced by the cleavage of the substrate luciferin by luciferase. We attempted to define conditions that would allow a "glow" luminometer to accurately measure hGH levels using a chemiluminescence assay that is based on the emission of light at 420–430 nm, accompanying the conversion of an acridinium ester (covalently linked to a secondary anti-hGH antibody), into *N*-methyl acridone. We used the Turner Model 20e luminometer (Turner Designs, Sunnyvale, CA, USA), which detects light at a peak of 420 nm and which has been shown to afford superior sensitivity among commercially available integrating luminometers (3).

hGH standards provided in the chemiluminescence assay kit were dissolved in distilled water and used to develop a standard curve using the Model 20e luminometer. Fifty microliters of each of the standards were incubated with glass beads containing an anti-hGH antibody directed against one hGH epitope, and a soluble anti-hGH

Table 1. Examination of the Stimulatory Effect of 1,25-Dihydroxyvitamin D3 (1,25-D3) on ROS 17/2.8 Stably Transfected with -523 bp of the Rat Osteocalcin 5'-Flanking Region Linked in Tandem to the hGH Gene; a Comparison of Responsiveness to 1,25-D3 When Transfected Cells are Cultured in the Absence or Presence of Serum

| | hGH, ng/mL | |
|--------------------|------------|------------------------------------|
| | Control | + 2.5 × 10 ⁻⁹ M 1,25-D3 |
| Cells + serum | 32 | 32 |
| Cells (serum-free) | 388 | 1028 |

antibody directed against a second hGH epitope. In the kit, an acridinium ester is covalently attached to the soluble anti-hGH antibody. After an incubation period of 1 h, the glass beads were washed as per the chemiluminescence protocol instructions and then transferred to a borosilicate glass culture tube with a plain end (10 × 50 mm, Cat. No. 14-962-22; Fisher Scientific, Pittsburgh, PA, USA). Other brands of borosilicate tubes yielded unacceptable background readings. The Turner Model 20e luminometer was then linked to a Cavro Model 2000 dispenser (Turner Designs) according to the manufacturer's instructions. Just prior to reading the samples, equal volumes of trigger 1 and trigger 2 solutions were mixed together. The parameter settings for the luminometer were then set as follows: Pre-Delay, 0 s; Delay, 5 s; Integrate, 0 s; Display, fixed range of 0.000. A borosilicate tube containing a glass bead was placed into the sample chamber, and the dispenser was set to deliver 150 µL of the mixture of the two trigger solutions. After delivery of the solution, the peak (P) value of the relative light units (RLU) was recorded. Prior to plotting the data, the background

value, representing the RLU of a glass bead plus 0 ng/mL hGH, was subtracted from each peak value. These corrected peak values were then plotted on logarithmic/logarithmic graph paper vs. hGH concentration (ng/mL). As can be seen in Figure 1, there is a linear relationship between the amount of hGH present in a sample and the amount of RLU detected. Upon the addition of a new sample into the sample chamber, 15 s were allowed to pass after the injector stand had been lowered onto the sample chamber prior to subsequent addition of the trigger mixture.

To demonstrate the utility of this assay system, a plasmid construct, consisting of -523 bp of the rat osteocalcin 5'-flanking region linked in tandem to the hGH gene (7), was stably transfected into the rat osteoblastic osteosarcoma cell line ROS 17/2.8 (4) using the calcium phosphate precipitation method (2). A 1,25-dihydroxyvitamin D3 (1,25-D3) response element is found within this 5'-flanking region, such that when exposed to 1,25-D3, stimulation of gene expression is observed. The transfected cells were plated into Falcon® 12-well plates (4 cm²/well) in 1 mL of medium contain-

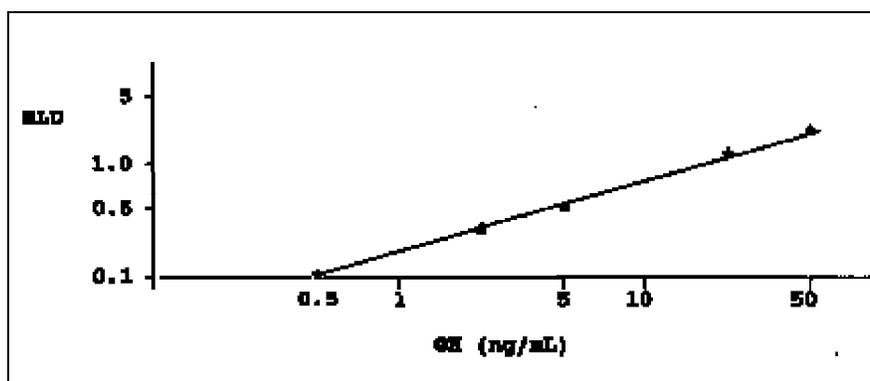


Figure 1. Plot of hGH concentrations vs. RLU. A plot on logarithmic/logarithmic scales of increasing concentrations of hGH (0.5, 1.6, 5.0, 16.0 and 50 ng/mL plotted on the x-axis) vs. RLU (y-axis) as determined using a Turner Model 20e luminometer. Each point is the average of samples assayed to triplicate.

ing 10% fetal calf serum. Two hours after plating, the medium was removed from one group of plates and replaced with 1 mL of serum-free medium. A portion of the plates from both conditions were then treated with 2.5×10^{-9} M 1,25-D3 and the rest remained untreated. Seventy-two hours later, the medium was collected from the plates and 50 μ L were assayed for hGH content as described above. As seen in Table 1, untreated cells in the presence of serum synthesized less hGH than did cells in serum-free medium and did not respond to 1,25-D3, whereas cells in the serum-free condition did respond. Based on the results of this experiment, all subsequent experiments using these cells would be performed in the absence of serum.

This report is intended to demonstrate that the Turner Model 20e, and potentially other "glow" luminometers, can be utilized as a "flash" luminometer under the parameter settings outlined. This report may be of particular value to those investigators who are using both luciferase and hGH as reporter genes. In addition, many other chemiluminescence assay kits are also available to measure the concentrations of hor-

mones, such as parathyroid hormone, thyroid-stimulating hormone, human chorionic gonadotropin, and it is reasonable to assume that these kits could also be used to accurately quantify the appropriate hormone concentration using a Turner Model 20e or possibly other "glow" luminometers.

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Funds to conduct this project were from Public Health Service Grant NIH AR41581-03 (P.T.G.). The authors acknowledge the helpful suggestions of Jim McCormick of Turner Diagnostics in determining the parameters for using the Turner Model 20e luminometer as a "flash" luminometer. Address correspondence to Peter T. Guidon Jr., Department of Biology, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, USA. Internet: guidonpe@lanmail.shu.edu

Received 25 January 1995; accepted 1 September 1995.

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