TECHNICAL NOTE

Multilabel Detection

Normalization of Luminescence Assays Using Glowell Microplate Standards, ATPLite Luminescence Assay Kit and EnVision Multilabel Plate Readers

Introduction

Luminescence assay technology is based on the detection of light produced by certain chemical reactions taking place in the sample. PerkinElmer's luminescence assay systems offer exceptional efficiency, speed and simplicity for research and drug discovery applications. However, since the measurement data obtained from the instrument is given in relative light units, it is very difficult to normalize and compare data obtained from different instruments and experiments.

The Glowell® microplate standards from PerkinElmer are a range of highly reliable luminescence standards which can be used to normalize data from different instruments and experiments as well as convert relative light units into absolute light units. All Glowell® standards come with a calibration certificate which is traceable to international standards.

The purpose of this technical note is to demonstrate how to use the Glowell® standards in order to normalize data obtained from two separate instruments, and how to convert the relative light units given by the instrument into absolute light units. For this purpose we have chosen to use the ATPLite® Luminescence Assay Kit and two EnVision® Multilabel Plate Reader instruments.

The ATPLite assay system is based on the production of green-yellow light from the reaction of adenosine triphosphate (ATP) with added firefly luciferase and D-luciferin. The ATPLite assay is used for quantifying the ATP concentration in the sample.

The Glowell® normalization method described here is not restricted to this particular choice of assay (ATPLite) and instrument (EnVision); it can therefore be applied to other instruments and luminescence assays.

PerkinElmer®
For the Better

Materials and methods

The ATP-standard 1/2-log dilution series was created using the ATP-Lite-M kit (PerkinElmer, 6016941, 6016943, 6016947, 6016949). The ATP dilution series (10^{-4}-10^{-13} M ATP) was made according to the instructions in the kit insert, and the samples were pipetted into wells of a white OptiPlate™-96 (PerkinElmer, 6005290). After preparation, the microplate was shaken for five minutes in an orbital shaker at 700 rpm, and subsequently measured (after 30 s dark adaptation) in two separate EnVision (models 2102 & 2103) readers, hereafter referred to as Env #1 (EnVision model 2102) and Env #2 (EnVision model 2103). Both EnVision instruments used the (factory preset) ultra-sensitive luminescence (US LUM 96) measurement protocol.

Two separate Glowell® (yellow) 96-well microplate standards (PerkinElmer, 1008-0040) were used to prepare two separate Glowell® normalization plates, hereafter referred to as Glo #1 and Glo #2 (i.e. one plate for each EnVision instrument). In each normalization plate (white OptiPlate-96) the three Glowell® samples (there are three Glowell® samples per kit, covering two orders of magnitude in light intensity) were placed in three separate wells. The Glo #1 and Glo #2 plates were measured with Env #1 and Env #2, respectively, using the same (factory preset) ultra-sensitive luminescence (US LUM 96) measurement protocol as in the case of the ATP dilution series.

The reason for choosing the yellow Glowell® standard for the normalization measurements is due to the fact that the luminescence emission spectrum of the yellow Glowell® standard (emission peak at ~ 560 nm) closely matches the luminescence emission spectrum of the ATPLite assay. Thus, similar instrument response would be expected for the yellow Glowell® standard and the ATPLite assay, leading to more accurate normalization results.

Results and analysis

The ATPLite measurement results are shown in Figure 1a (Env #1) and Figure 2a (Env #2), and the Glowell® measurement results are shown in Figure 1b (Env #1, Glo #1) and Figure 2b (Env #2, Glo #2). Since the light output from the Glowell® samples have an intrinsic decay, due to the radioactive decay of tritium together with the degradation of the luminous coating, the (radiant) photon flux F (photons/s) of the Glowell® samples (x-axes in Figures 1b and 2b) at the time of the measurement with the EnVision instruments were calculated using Equation 1.

$$F = F_0*(1-k*(t-t_0)) \quad (1)$$

t_0 = Calibration date (time) of the Glowell® sample (found in the Glowell® calibration certificate).

F_0 = Calibrated photon flux value of the Glowell® sample (found in the Glowell® calibration certificate).

t = Date (time) when the Glowell® sample is measured.

F = Photon flux of the Glowell® sample at the time of measurement.

k = 0.000333 = Glowell® sample (light output) decay rate (found in the Glowell® product sheet. Since k has the unit [1/days] the difference $t-t_0$ must be given in days).

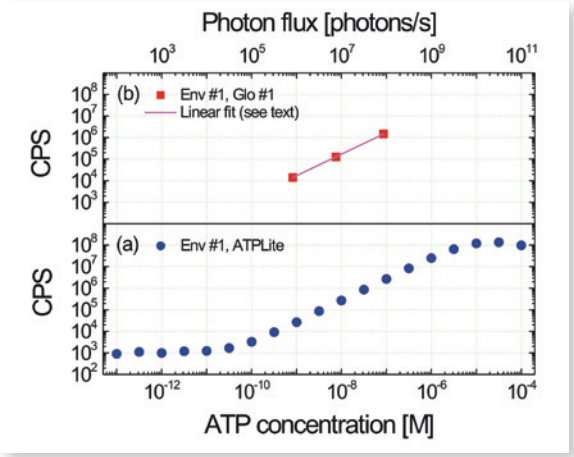

Figure 1. (a) ATPLite dilution series measured with Env #1. (b) Glo #1 normalization plate measured with Env #1. CPS = counts/s.

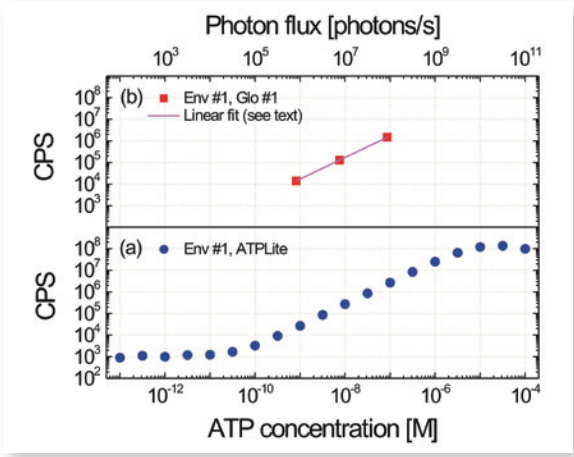

Figure 2. (a) ATPLite dilution series measured with Env #2. (b) Glo #2 normalization plate measured with Env #2. CPS = counts/s.

Table 1 shows the measurement results and the difference between the two EnVision instruments for the six ATP concentrations in the interval $3.16*10^{-10}$ – 10^{-7} M, corresponding to the region ~10^4 – $3*10^6$ CPS where the ATPLite counts overlap with the Glowell® counts and the instrument response is linear. From the results it is clear that the Env #2 instrument gives ~ 4-7% larger values than the Env #1 instrument.

In order to normalize the results from the two EnVision instruments, the ATPLite data was converted from relative light units (counts/s = CPS) into absolute light units (Photon flux = photons/s = F) using the Glowell® data. This was done by calculating a linear fit (see solid lines in Figures 1b and 2b) to the Glowell® data using Equation 2. For each of the three (i = 1,2,3) Glowell® data points (see Figures 1b and 2b) the slopes $c_i = CPS_i/F_i$ were calculated, and then averaged to yield the final slope. The results are shown in Table 2. It must be noted here that a standard (no weighting) least squares fit would put too much "weight" on the highest data point, and thus the overall results would be less accurate than in the method described above.

$$CPS = c * F \quad \leftrightarrow \quad c = CPS/F \qquad (2)$$

Using the average slopes c(ave), the data (counts) in Table 1 was converted into absolute light units (photons/s) by dividing the counts with the corresponding slope for the instrument (i.e., F = CPS/c). The results can be seen in Table 3, together with the calculated difference between the instruments (a positive/negative percentage means the Env #2 value is larger/smaller than the Env #1 value). The results clearly show that the difference between the absolute values is much smaller (average difference less than 1%) than the corresponding difference between the relative values in Table 1. Thus, after normalization the measurement values obtained with the two EnVision instruments are on average within 1% of each other, a marked improvement in accuracy.

To convert and compare the relative counts between the instruments, the conversion factor is given by the ratio of the two average slopes in Table 2:

CPS(Env #2) = (0.017794/0.017023) * CPS(Env #1) = 1.0453 * CPS(Env #1)

Table 1. **Comparison of ATPLite measurement values (before normalization with Glowell standard) for the two EnVision instruments.**

ATP conc. [M]	Env #1 [CPS]	Env #2 [CPS]	Difference [%]
3.16E-10	9156	9502	3.8
1.00E-09	26471	27853	5.2
3.16E-09	85920	89422	4.1
1.00E-08	268049	278653	4.0
3.16E-08	853067	893267	4.7
1.00E-07	2663556	2852769	7.1

Table 2. **Calculation of linear fit to Glowell data points in Figures 1b and 2b.**

	F [photons/s]	CPS [counts/s]	c = CPS/F [counts/photons]	c(ave) [counts/photons]
Env #1	8.23E+05	14072	0.017092	
Glo #1	7.57E+06	127424	0.016831	0.017023
	8.57E+07	1469064	0.017144	
Env #2	1.16E+06	19616	0.016964	
Glo #2	1.26E+07	223956	0.017842	0.017794
	1.55E+08	2882444	0.018576	

Table 3. **Comparison of ATPLite measurement values (before normalization with Glowell standard) for the two EnVision instruments.**

ATP conc. [M]	Env #1 [photons/s]	Env #2 [photons/s]	Difference [%]
3.16E-10	537850	534014	-0.7
1.00E-09	1555064	1565327	0.7
3.16E-09	5047431	5025431	-0.4
1.00E-08	15746723	15660012	-0.6
3.16E-08	50114008	50200608	0.2
1.00E-07	156472465	160322486	2.5

Conclusion

Glowell® microplate standards were used to normalize ATPLite luminescence data obtained using two EnVision instruments. Before normalization the relative counts measured by the two instruments showed a ~ 5% difference. Using the Glowell® standards the relative light units (counts/s) given by the instruments were converted into absolute light units (photons/s). The resulting normalized data showed a difference less than 1% for the two instruments, a marked improvement over the original (relative counts) data. Thus, we have shown that the Glowell® microplate standards provide an efficient and accurate way of normalizing luminescence data obtained with microplate readers.

Author

Markus Westerling, Ph.D.

PerkinElmer, Inc.
Turku, Finland

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

008025A_01 Aug 2012 Printed in USA