TopCount *Topics*

TCA-023

High Throughput Luminescence Assays Using Six Detectors

Abstract

The TopCount™ Microplate Scintillation and Luminescence Counter has been widely used for ultra-sensitive, single photon, luminescence counting. The measurement of two wells simultaneously and the automatic processing of 30 or more microplates has made the TopCount ideal for rapid, accurate, and sensitive luminescence counting.

The growing use of luminescence assays in high throughput screening programs has led to the enhancement of multiple detector luminescence counting capabilities on the TopCount. Six simultaneously counting detectors allow extremely accurate and rapid processing of luminescent samples. Use of this feature in conjunction with stable luminescent chemistries permits the unattended and automated processing of many thousands of samples in a day. Studies described below using advanced, long half-life chemistries demonstrate the performance of this high throughput method.

Introduction

Functional assays based on genetic regulation and cellular growth have become an important part of high throughput screening programs for pharmaceutical development. Examples include reporter gene assays, where gene regulation is assessed by the production of enzymes that can be detected directly or indirectly, and cell proliferation assays, where radiolabeled thymidine is incorporated into replic ating strands of DNA. New instrumentation, such as the TopCount and the FilterMate™ Universal Harvester, have permitted the development of true high throughput screening programs based on the above applications, where thousands of samples can be assayed in microplates unattended.

The interest in non-separation (homogeneous) assays has led to the development of non-isotopic, luminescence assays which are safer to use and which do not require separation steps. One example is LucLite™ (Packard Instrument Company), a reporter gene assay based on the production of luciferase which emits a photon when triggered by an appropriate substrate.[1] An example of a cell viability assay using luminescence technology is CytoLite™ (Packard Instrument Company), in which the luminescent signal is proportional to the number of cells in a growing culture.[2] Both of these assays produce signals that are stable over long periods of time. The TopCount is well suited for these assays because of its low background and high photon counting dynamic range. With a background of approximately 20 counts per second (CPS) and an accurate measurement of over 20 x 10^6 CPS, TopCount has a 10^6 dynamic range. In addition, two or six detectors and a stacker enhance throughput for multiple-plate assays.

Recent advances in electronic technology have dramatically enhanced the suitability of TopCount for luminescence-based functional cell assays. These advances now allow the simultaneous luminescence counting of up to six samples with the same low background and high dynamic range. With six detectors counting simultaneously, it is possible to count an entire microplate in less than one minute. Detector normalization ensures that the results from each detector are within +/- 5% of the other detectors. The capability to read up to six wells at the same time permits the assaying of a far greater number of microplates in a given period of time. In addition to overall gains in screening efficiency, this feature allows true multiplate processing of luminescence samples having limited signal durations. For example, the Luciferase Assay System™ (Promega

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Corporation) produces a luminescence signal which lasts only a few minutes.[3] In a single or dual detector system, it is only possible to process one or two microplates before significant signal decay occurs.[4] With six simultaneously counting detectors on the TopCount, it becomes possible to measure many more microplates before signal decay becomes significant. Experiments detailed below demonstrate the performance of this feature.

Methods

Detector Uniformity

In any multiple detector system, it is critical that each detector produces the same results for a series of similar samples. The six luminescence detectors on the TopCount are normalized using a stable luminescent source to ensure that each will produce comparable results. To evaluate detector uniformity, a stock solution of CytoLite activator solution[2] was prepared by diluting 10x in water. Twenty microliters of the diluted activator was added to each well of a 96-well white microplate (PicoPlate™, Packard Instrument Company), followed by 180 µL of ethanol, which produces a stable luminescent signal. The plate was sealed with TopSeal-A™ self-adhesive cover film and counted on the TopCount in the forward orientation for 0.1 minutes per well using all six detectors. For low backgrounds or very high luminescence CPS, black LitePlates™ (Packard Instrument Company) can be used.

Linearity and Sensitivity

Both CytoLite and LucLite were used to evaluate signal linearity and sensitivity for each detector over a series of concentrations. For CytoLite, a series of dilutions of the activator solution was prepared ranging from 1x to 0.01x. Twenty microliter aliquots of the appropriate dilution were dispensed into wells of a white polystyrene microplate (OptiPlate™, Packard Instrument Company) such that six columns (one for each detector) on the plate contained a full dilution series plus an assay blank. Luminescent signal was triggered in each well by the addition of 180 µL ethanol. The plate was sealed and counted in the TopCount as previously detailed.

For LucLite, a stock solution of luciferase was diluted in 1 mM $MgCl_2$ from a concentration of 500 ng/mL to 2.5 ng/mL. Ten microliter aliquots of the diluted luciferase were dispensed into wells of an OptiPlate in the manner described above. The LucLite substrate containing luciferin was reconstituted according to the kit instructions and 100 µL added to each well containing luciferase. Blank wells (luciferin minus luciferase) for each detector were included. The plate was sealed and counted in the TopCount as previously detailed.

Results

Detector Uniformity

The TopCount measures microplate wells in a column-wise manner, with each detector measuring a set of two columns containing a total of 16 samples each. Table 1 summarizes the counting results of the PicoPlate containing 96 identical samples of the CytoLite activator solution. Each detector's average CPS is within +/- 4% of the overall average CPS. This is well within the variation expected from pipetting and confirms that the TopCount's detectors are normalized to give a minimum of variation.

Detector	Average CPS n = 16	Average CPS n = 96	% Difference from Overall Average CPS
1	143480	144158	-0.47
2	143499	144158	-0.046
3	143018	144158	-0.79
4	140394	144158	-2.61
5	144762	144158	0.42
6	149794	144158	3.91

Table 1.
Analysis of detector uniformity for 96 luminescence samples. Uniformity calculated as percent difference from overall average counts per second (CPS).

Linearity and Sensitivity

After measuring the signal produced by identical serial dilutions of the CytoLite activator solution, dilution curves were constructed for each detector and superimposed on one another (Figure 1). These results show that, over a wide range of count rates, each detector will give the same results as all of the others. Moreover, signals measured by one detector can be accurately compared to those measured by another. This means that only a single calibration curve is required per assay.

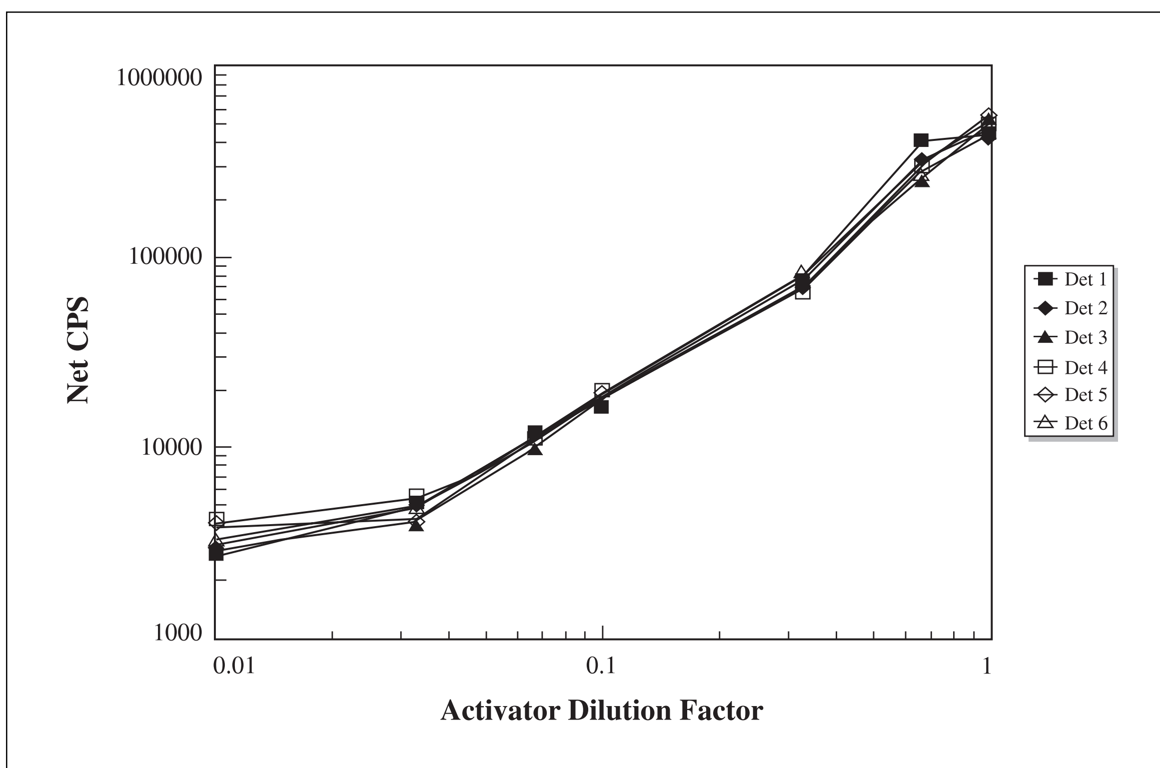

Figure 1.

Serial dilutions of CytoLite activator measured with each of six detectors on TopCount. Net CPS values are calculated by subtracting an assay blank composed of 20 µL deionized water and 180 µL ethanol.

Serial dilutions of luciferase were measured using the LucLite reporter gene assay kit. The raw data from each of the six detectors are listed in Table 2. For the dilution series as a whole, the C.V. value averages 6.6%, and no single value exceeds 10%, even at low count rates. Given that these results are spread over a dynamic range of greater than four orders of magnitude, the variation between detectors is not significant. Figure 2 shows the results graphically. As with the CytoLite assay, the results demonstrate that over a wide linear dynamic range, each of the six detectors produces a statistically identical dilution curve. In this experiment, 2.5 pg of luciferase was accurately detected, although by optimizing the protocol, as little as 10 fg luciferase can be detected using LucLite.

Luciferase pg	Det. 1	Det. 2	Det. 3	Det. 4	Det. 5	Det. 6
5000	1640078	1532994	1417141	1716459	1651383	1509157
2500	564717	483063	547206	491971	546373	534648
500	51121	52605	58506	56860	56864	54235
250	20571	21736	22607	22916	21773	20859
50	2835	2120	2385	2342	2197	2215
25	1069	893	982	917	968	900
5	177	174	187	186	173	165
2.5	73	78	84	78	78	63
Blank	41	51	51	45	42	41

Table 2.

CPS of luciferase dilutions measured on TopCount using six detectors. Blank samples consist of 10 uL 1 mM $MgCl_2$ + 100 µL reconstituted LucLite substrate.

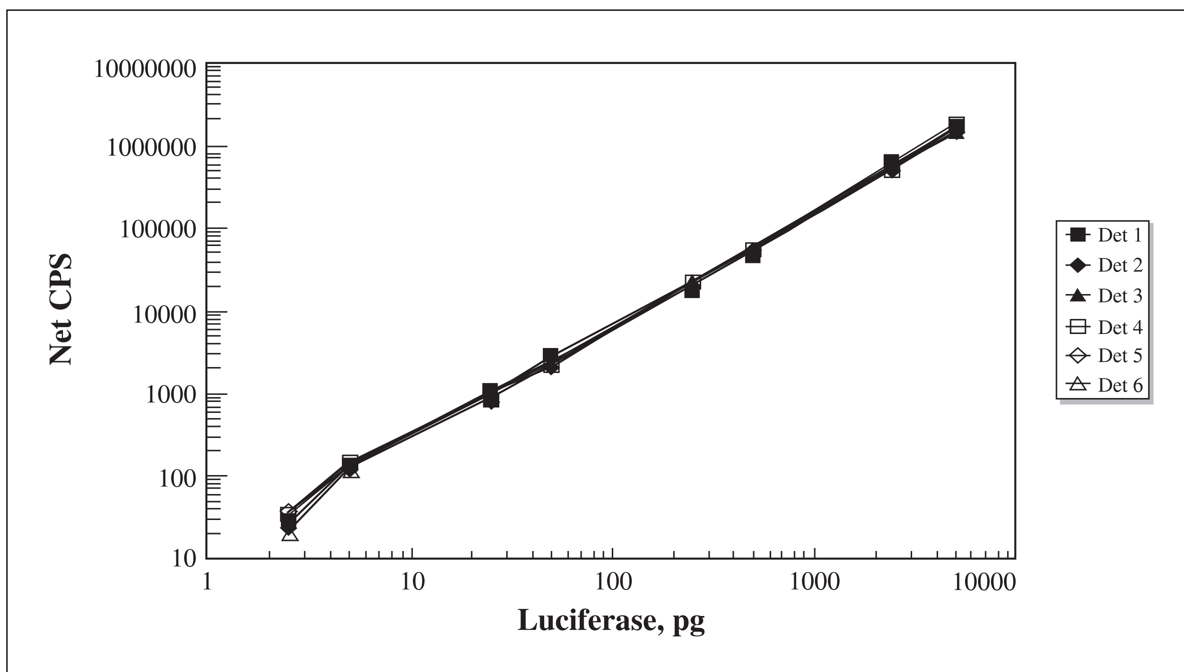

Figure 2.
Dilution curves of luciferase measured with LucLite kit on TopCount using six detectors.

Conclusions

Ultra high throughput luminescence assays have been made practical with the advent of multidetector luminescence counting using the TopCount Microplate Scintillation and Luminescence Counter. Six simultaneously counting detectors allow the accurate quantitation of 96 luminescence samples in less than one minute. Advanced photon counting technology produces accurate results over six orders of magnitude, with inter-detector variations of less than +/- 5%. This application, along with existing TopCount features such as open software and hardware architecture, permit easy automation of true high throughput luminescence assays with the use of advanced laboratory robotic systems.

References

1. LiteGuides #2 (1994). Introduction to LucLite™; A Bioluminescent Reagent System for Reporter Gene Assays. Packard Instrument Company.

2. LiteGuides #1 (1994). Introduction to CytoLite™; A Chemiluminescent Reagent System for Cell Proliferation and Cytotoxicity Assays. Packard Instrument Company.

3. Technical Bulletin #101, Luciferase Assay System, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399.

4. TopCount Topics #14 (1992). The Measurement of Luminescence. Packard Instrument Company.