High-Throughput Automation of a Dual Reporter Assay in Low Volume 384 & 1536 Well Plate Formats using Deerac Fluidics Equator™ NS808 – Eight Tip Pipetting system, Promega's Chroma-Luc™ Technology and BMG LABTECH's PHERAstar Microplate Reader.

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1. ABSTRACT

The drive towards miniaturization within the pharmaceutical and biotechnology fields has created a need for liquid handling technologies that accurately deliver low volume reagents to high-density plates. This has also created a need for simple, fully scaleable assays in low volumes. Here we demonstrate the successful combination of both through the use of the Equator™ NS 808 - Eight Tip Pipetting System and the dual color Chroma-Luc™ technology. Cellular lysates, containing the green CBG99*luc* and red CBR*luc* genes, followed by Chroma-Glo™ reagent, were dispensed in low-volume 384 and 1536 well formats in volumes ranging from 10ul to 500nl. Luminescence from the two luciferases was then simultaneously measured using the BMG LABTECH PHERAstar plate reader. The exceptional Z' Factor scores, linearity, limit of detection, and separation of signal data, shows the flexibility and reliability of the Equator™ NS 808, and Chroma-Luc™ dual reporter technology in any high-throughput situation.

2. INTRODUCTION

HTS Adaptable Chemistries

The Chroma-Luc™ technology consisting of the Chroma-Luc™ Series Reporter Vectors and Chroma-Glo™ Luciferase Assay System represents a chemistry ideally suited for high-throughput and ultra-high-throughput screening. Each assay procedure requires only a single reagent addition to the well. The homogeneous format also allows for miniaturization of the assay provided that the one-to-one ratio of reagent to culture medium is preserved. The short incubation times and extended luminescent signal half-lives of the chemistry creates straightforward processing of plates, both in continuous and batch mode formats.

Automated Non-Contact High-Throughput Liquid Dispensing

The use of high density, low-volume plate formats has created the need for pipetting systems capable of rapidly delivering reagents in a consistent fashion across increasingly smaller volume ranges. To meet these demands, screening facilities have turned to non-contact liquid handling systems that can deliver reagents to a plate in seconds, and can repeat this process for hundreds of plates without the risk of cross-contamination across wells. In order to test Promega's Chroma-Luc™ technology using this type of dispensing, the Deerac Fluidics Equator™ NS-808-Eight Tip Pipetting System (Table 1, Figure 1, 2) was used to pipette cell lysates, and reagents to the assay plates. Using the spot-on™ dispense technology from Deerac Fluidics, the Equator™ dispensed a broad volume range in non-contact mode. This was achieved through simple method set-up in the software; the instrument requires no hardware changes at all. Table 2 shows CVs for all assays performed in low-volume 384-well, and 1536-well formats. CVs were less than 10% in total assay volumes as low as 2µl.

Volume range	50nL-20µL
Volume increment	Freely adjustable
Speed	<15s (1536 well plate) <30s (96 well plate)
Accuracy	< 10% @ 50nL < 10% @ 1,000nL
Precision	< 10% @ 50nL < 5% @ 1,000nL
Viscosity range	0.5 - 6.0 cP
Plate formats	96, 384, 1536 & custom

Table 1. Equator™ NS 808 liquid handling product specifications

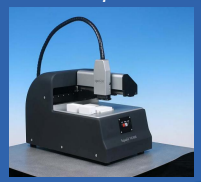

Figure 1. Equator™ NS 808

Figure 2. 1536 format dispense onto a flat surface.

Chemistry	Assay Format	Assay Volume	Lysate	Cell Conc.	% CV
Chroma-Glo™	LV384	20ul	Red	5000	4.14%
Assay System			Green	5000	3.47%
		10ul	Red	2500	5.01%
			Green	2500	6.10%
	1536	8ul	Red	2000	5.65%
			Green	2000	5.95%
		5ul	Red	1250	5.35%
			Green	1250	4.36%
		2ul	Red	500	8.38%
			Green	500	6.39%

Table 2. % CV data for Chroma-Glo™ assay system. Cell concentrations based on 250 Cells/ul standard. Cell lysates and reagents were dispensed with Deerac fluidics Equator ™ NS 808 and read with a BMG LABTECH PHERAstar reader.

HTS/uHTS Microplate Analysis

To qualify Promega's Chroma-Luc™ technology using instrumentation commonly found in screening facilities, we ran Chroma-Glo™ assays in low-volume 384-well and 1536-well formats, and analyzed the plates using the BMG LABTECH PHERAstar Microplate Reader. The PHERAstar offers highly accurate, sensitive reading capabilities in 96-, 384-, and 1536-well plates, across a wide variety of detection modes. This reader also provides simultaneous dual-emission Microplate reading, which makes this instrument ideal for reading the red and green Chroma-Luc™ luciferase signals.

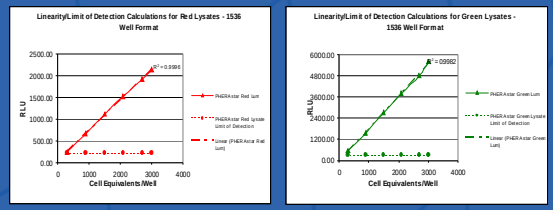

Figure 3. Graphs showing linearity and sensitivity of Chroma-Glo™ chemistry, using red (CBRluc) and green (CBG99luc) luciferases. 300-3000 Cell equivalents tested with assays run on Deerac Fluidics Equator™ NS-808 and read using the BMG LABTECH PHERAstar. All assays performed with an 8ul total volume in 1536 well format

3. TESTING AND RESULTS

Chroma-Luc™

Chroma-Luc™ technology evaluations were performed in order to test assay sensitivity and linearity for red and green luciferases, using lysates at various cell equivalent concentrations (Figure 3). Red (*CBRluc*) luciferase, and green (*CBG99luc*) luciferase lysates were used for these evaluations. The lysates were prepared by the addition of Glo-Lysis Buffer (Cat.# E2661) to CHO cells stably-transfected with either *CBRluc* or *CBG99luc* genes. Assays were run in an 8ul total volume, in 1536-well format. Limit of Detection was calculated as the difference between sample averages and background plus three standard deviations.

Separation of the two luminescent signals was also assessed using various combinations of the red and green luciferase lysates (Figure 4). Lysates dispensed using the Deerac Fluidics Equator™ NS-808-Eight Tip Pipetting System were dispensed at 100/0, 90/10, 70/30, 50/50, 30/70, 10/90, and 0/100 percent concentrations of red/green luciferase lysate, respectively. These plates were analyzed using the BMG LABTECH PHERAstar Microplate Reader. Determination of assay robustness was calculated through the use of Z'-Factor scores (Table 3). Induced and uninduced, cotransfected, Chroma-Luc™ vectors were used for this purpose, as well as to ascertain fold induction values with this chemistry. d293 Cells were cotransfected with pCRE-CBG99*luc* and pCRE-CBR*luc* genes. The cells were then treated with either Isoproterenol HCl(1uM)/RO(100uM) or RO(100uM) at 24hrs. post-transfection.

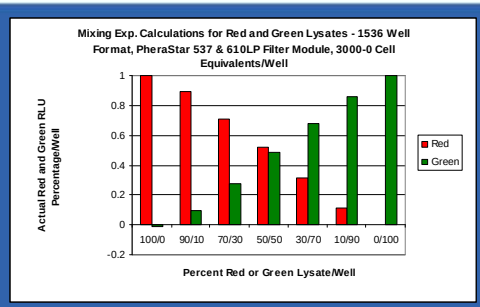

Figure 4 Graph showing comparison of actual separation of red and green luminescent signals to ideal separation percentages. Lysates dispensed using the Equator NS808 and analysed using the BMG LABTECH PHERAstar were dispensed at 100/0, 90/10, 70/30, 50/50, 30/70, 10/90 and 0/100 percentage concentrations of red and green luciferase lysate respectively. Ideal RLU percentages equal the percent of red and green lysate added per well

At 3 hrs. post-treatment, media was removed, and cells were lysed with 20ul Glo-Lysis Buffer, and frozen at –80C. Induced and uninduced lysates were pooled and used for all Z'-Factor and fold induction determinations. Lysates and reagent were dispensed in 20ul and 10ul total volumes in low-volume 384-well format, or in 8ul, 5ul, and 2ul total volumes in 1536-well format. Pipetting of lysate and reagent to assay plates was completed using the Deerac Fluidics Equator™ NS-808-Eight Tip Pipetting System. Relative light units were detected using the BMG Labtech PHERAstar Microplate Reader, containing a LongPass 537 and LongPass 610 filter module.

Well Format	Assay Volume	Cell Equiv. Per Well	Inst. Filter	Z'-Factor Value	Fold Induction
384	20ul	10,000	Red	0.84	14.88
			Green	0.86	13.37
	10ul	5,000	Red	0.81	13.84
			Green	0.77	13.17
1536	8ul	4,000	Red	0.75	12.54
			Green	0.72	7.07
	5ul	2,500	Red	0.78	15.05
			Green	0.82	12.35
	2ul	1,000	Red	0.67	14.65
			Green	0.74	12.18

Table 3 Z'- factor and fold induction values for Chroma-Glo™ assays in low-volume 384 well or 1536 well formats. Assays run on the Equator NS808 were read using the PHERAstar from BMG LABTECH.

4. SUMMARY

The results shown here illustrate:

•The reliability and performance of the Equator low volume liquid handler, demonstrated by low CVs attained over a wide range of volumes tested.

• The flexible nature and adaptability of the Chroma-Luc™ technology.

• The high degree of linearity across wide ranges of cell lysate concentrations, in low-volume 384-well and 1536-well formats.

• Excellent Z'-Factor scores attesting to the robustness of liquid handling technology and assay chemistry in volumes and plate formats currently used in screening facilities.

Reference

1. Zhang, J. *et al.* (1999) *J. Biomol. Screening* **4**, 67–73.

New Horizons in Liquid Handling