Miniaturization of a Luciferase Reporter Gene Assay in 384 Well Format

Application Note

David Hayes, Yu Alice Gao, Michael Briggs and Todd Upton, Corning Incorporated, Life Sciences

Introduction

The luciferase reporter gene assay is widely used to study G-protein coupled receptor (GPCR) activities and for screening compounds that can enhance or inhibit such activities. When performed in 384 well formats, these assays often utilize normal volume (NV) white tissue culture microplates with total well assay volume ranging from 30 to 60 µL. Because these volumes can entail significant cost in high throughput screening applications, assay miniaturization is a useful cost reduction goal.

In this study, we present the performance data of a miniaturized luciferase reporter gene assay (SteadyLite HTS assay from Perkin Elmer®) with a transfected HEK 293 cell line, using Corning® 384 well white, flat bottom, low volume (LV) microplates (Cat. No. 3826). We miniaturized the to a total assay volume of 17.5 µL, while simultaneously improving signal to background ratio (S:B), assay window, and variation, when compared to NV microplates.

Materials and Methods

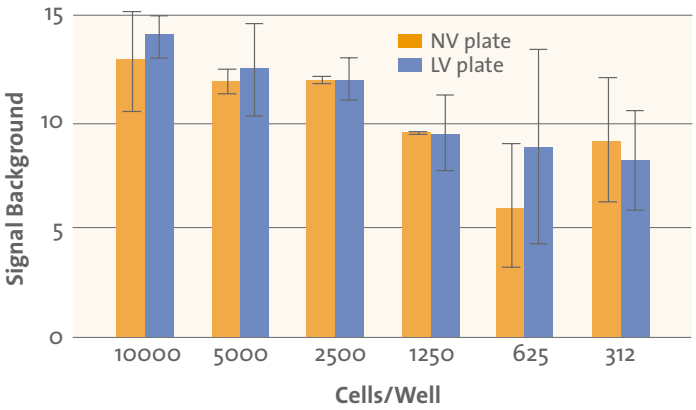
For all experiments, HEK 293 CRE-luc cells (Panomics RC0007) were propagated in IMDM without phenol red with 10% FBS and 50 µg/ml hygromycin; this cell line is stably transfected with a luciferin reporter gene driven by a promoter responsive to the CREB transcription factor. In preparation for this assay, cells were harvested from a culture flask using a Trypsin EDTA solution, and microplates were seeded at 2,500 cells per well in 10 µL of medium in Corning 384 well white LV flat bottom microplates (Cat. No. 3826). NV microplates (Cat. No. 3704) were seeded at 2500 cells per well in 20 µL of medium. Cells were induced with forskolin (Sigma Cat. No. F3917), to a final assay concentration of 20 µM, and incubated overnight in a humidity controlled incubator (37° C, 5% CO_2). Before reading, the microplates were equilibrated to room temperature for 30 minutes. Once the microplates had equilibrated, 5 µL of SteadyLite HTS (Perkin Elmer, Cat. No. 6016989) was added to each well of a LV plate for a final assay volume of 17.5 µL; whereas, per manufacturer's instructions, 25 µL of SteadyLite was added to each well of a NV plate for a final assay volume of 50 µL. The microplates were incubated at room temperature for 5minutes and read on an Acquest plate reader (Molecular Devices).

CORNING

Results

Several assay conditions were assessed: seeding density, impact of total assay volume and SteadyLite reagent usage, and cross plate performance. We first confirmed that both NV and LV microplates could be used with both 50 µL and 17.5 µL volumes using the SteadyLite assay (data not shown). Figure 1 shows the S:B ratio in a 50 µL assay volume over a titration of cell numbers in both microplates. The data indicates that reducing cell number per well to 2,500 cells/well does not reduce the S:B ratio over larger cell numbers. We therefore used this cell number in the remainder of the studies.

Figure 1. Impact of cell seeding density on signal to background ratio.



Total assay volume for each plate is 50 µL. NV microplates (Cat. No. 3704) (orange) and LV microplates (Cat. No. 3826) (blue) were used. Bars represent the average ± standard error for 2 experiments with 5 wells per condition.

We next set out to determine the lowest assay volume that generated robust and reproducible results by titrating the SteadyLite reagent. The data in Figure 2 indicate that using as little as 5 µL of SteadyLite reagent in a total assay volume of 17.5 µL is capable of lysing the cells and obtaining suffi-

cient signal intensity. Interestingly, using more SteadyLite reagent can have an adverse effect on overall signal intensity, likely due to the increased total assay volume (Figure 2).

Finally, inter- and intraplate performance was evaluated with the results shown in Table 1. The LV plate outperformed the NV plate in all categories investigated: (%CV, S:B ratio, and Z'). These observations demonstrate that along with allowing assay miniaturization, the LV plate can also improve data quality.

Conclusions

- Use of a Corning® low volume assay microplate can improve important cell based assay readouts such as % CV, S:B ratio, and Z' over standard normal volume microplates.
- A standard assay size of 50 µL can be reduced to 17.5 µL in both NV and LV microplates using the Perkin Elmer SteadyLite assay kit. The miniaturized assay can reduce reagent cost by >80%.

Table 1. Inter- and Intraplate Performance

	Normal Volume (50 µL)		Low Volume (17.5 µL)	
	Intraplate	Interplate	Intraplate	Interplate
%CV	8.78	12.11	6.29	10.43
S:B	10.99	11.03	12.04	11.95
Z'	0.73	0.66	0.80	0.70

n = 192 wells

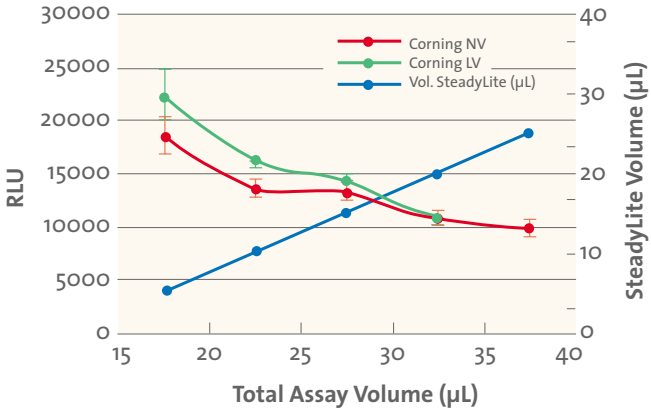


Figure 2. Effect of the volume of SteadyLite on total assay signal. An increasing amount of SteadyLite was added to an initial volume of 20µL of media. NV plates (Cat. No. 3704) (Red) and LV plates (Cat. No. 3826) (Green) were used. Data represent the average ± standard error for 2 experiments with 7 wells per condition.

CORNING

Corning Incorporated
Life Sciences
Tower 2, 4th Floor
900 Chelmsford St.
Lowell, MA 01851
t 800.492.1110
t 978.442.2200
f 978.442.2476

www.corning.com/lifesciences

Worldwide Support Offices

ASIA/PACIFIC

Australia
t 61 2-9416-0492
f 61 2-9416-0493

China
t 86 21-3222-4666
f 86 21-6288-1575

Hong Kong
t 852-2807-2723
f 852-2807-2152

India
t 91-124-235 7850
f 91-124-401 0207

Japan
t 81 (0) 3-3586 1996/1997
f 81 (0) 3-3586 1291/1292

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6733-6511
f 65 6735-2913

Taiwan
t 886 2-2716-0338
f 886 2-2716-0339

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands & All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

LATIN AMERICA

Brasil
t (55-11) 3089-7419
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589