Miniaturization of a Luciferase Reporter Gene Assay Shows Enhanced Assay Performance With Considerable Cost Savings

SnAPPShots
A brief report from the Corning Applications Group

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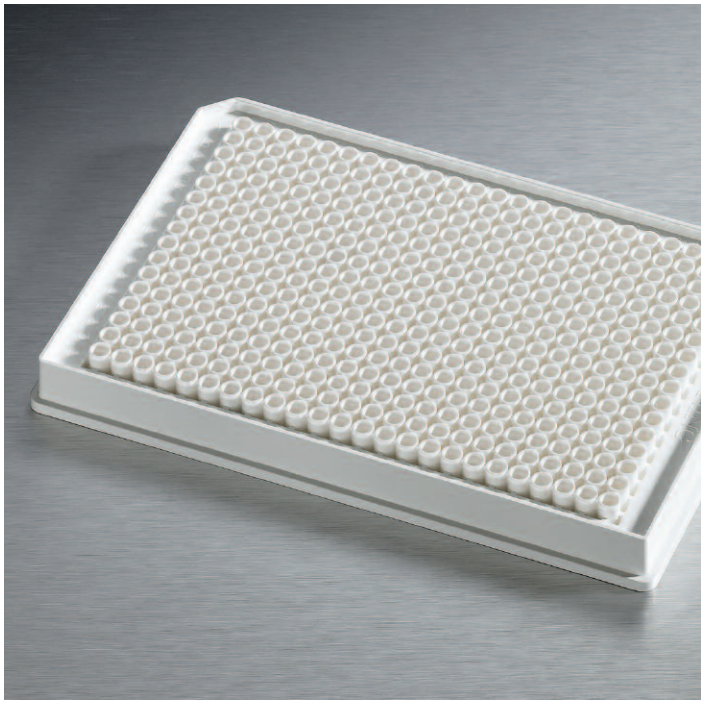
Introduction and Purpose

Assay miniaturization has become an important goal for scientists attempting to reduce costs associated with drug development. By moving to a Low Volume (LV) format one effectively reduces the waste associated with Normal Volume (NV) assays. Reducing assay volumes from 100 µL to 10 µL, like those found in 96 and low volume 384 well microplates respectively, can drop reagent costs linearly with volume (1). The purpose of this study is to demonstrate how miniaturizing a Luciferase reporter gene assay from a traditional 45 µL/well down to 17.5 µL/well utilizing the Corning® 384 well LV microplates impacts assay performance.

Methods and Results

Using the Corning 384 LV well tissue culture (TC)-treated flat bottom microplates (Cat No. 3826), HEK 293 CRE-luc cells (Panomics Cat. No. RC0007) were seeded at a density of 2,500 cells/well in either 10 (LV) or 20 µL (NV). The cells were cultured in IMDM without phenol red with 10% FBS and 0.1 mg/mL hygromycin. This cell line is stably transfected with a luciferin reporter gene driven by a promoter responsive to the cyclic AMP response element-binding (CREB) protein. The cells were induced with 20 µM forskolin in a volume of 2.5 or 5 µL by incubation overnight, in a humidity controlled incubator at 37°C with 5% CO_2. Prior to reading, the plates were equilibrated to room temperature for 30 minutes, after which 5 or 20 µL of SteadyLite HTS Luciferase reagent (Perkin Elmer™) were added to the appropriate wells and the plates read on a Molecular Devices Aquest® plate reader.



Corning 384 Well Low Volume Flat Bottom Microplate (Cat. No. 3826)

By miniaturizing the assay we observed a considerable cost savings in terms of reagent and compound use (refer to Table 1). The cost savings are realized by the 75% reduction in the SteadyLite reagent utilized in the LV assay which equates to a cost/well that is 24% of a NV plate. There was also minimal impact on the Z' score (Figure 1) and similar, if not higher, signal to background ratio in the LV versus NV assay plates (Figure 2). The geometry of the wells of Corning's 384 well tissue culture (TC)-treated flat bottom microplates functions not only to allow cost savings but also to concentrate the signal in the LV assay and improve the S:B ratio.

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Figure 1. Z' Score

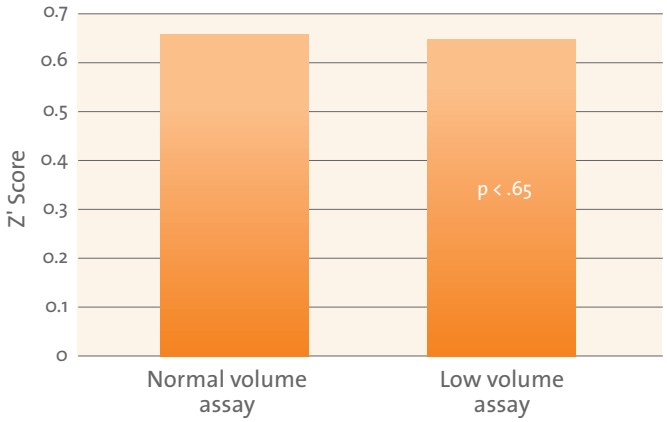
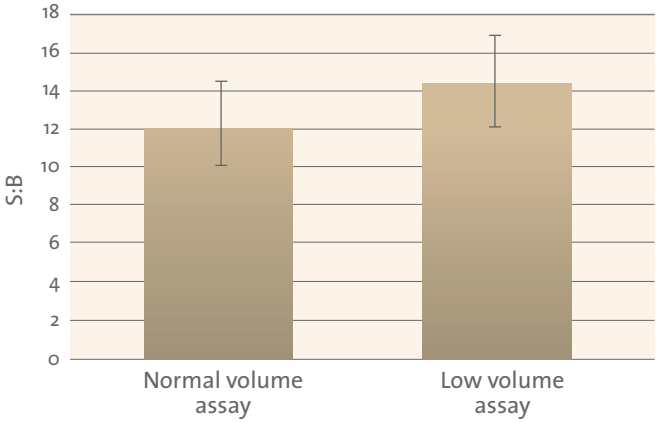


Figure 2. S:B



Conclusions

- Miniaturization leads to a 75% reduction in SteadyLite usage as well as a considerable cost savings using the low volume assay format.
- Assay robustness in LV plates is equal to or better than that seen in NV plates.
- The overall geometry and LV format equate to both cost savings and enhanced assay performance.

Reference

1. Tina K. Garyantes, 1536-well assay plates: when do they make sense? DDT Vol. 7, No. 9 May 2002 editorial.

Table 1.

	Normal Volume Assay	Low Volume Assay
mL of SteadyLite/kit	1000	1000
µL SteadyLite/well	20	5
Number of wells/1000 mL	50000	200000
List price of reagent kit	$2,688	$2,688
Cost per well	0.054	0.013

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