



Miniaturization and Automation of a Luciferase Reporter Gene Assay

Abstract

In drug discovery, cost savings is a constant goal. Direct labor and reagents represent two of the costliest aspects of a high throughput screen. These costs can be mitigated by assay miniaturization and automation. The luciferase reporter gene assay has become a widely used tool in studies of G-protein coupled receptor activities, and for screening compounds that regulate receptor activities. In this application note, we present performance data and a cost savings estimate for a miniaturized and automated luciferase reporter gene assay using a transfected HEK 293 CRE-luc cell line. Miniaturization is accomplished using Corning® 384 well low volume microplates. Automation is accomplished with a unique, low cost, automated system using the Caliper Sciclone ALH 3000 and a Liconic® STX 40 automated incubator. Through full automation and assay optimization, we achieved an 80% reduction in reagent cost in a 24 hour operation, and maintained Z' and signal to background ratios equal to or better than those achieved by hand at normal volume.

Introduction

The goal of this work was twofold, firstly, to miniaturize and automate a reporter gene assay, and secondly, to design a system that was both simple and cost effective for working with cells. The miniaturization was accomplished by using low volume 384 well microplates from Corning. Although automation can provide excellent day-to-day precision and accuracy, it was also important to compare the automated liquid handling results to manual results, to know whether they are comparable. For the automated liquid handling we used the Sciclone ALH 3000. However, in addition to liquid handling, incubation of the cells was required, and so we directly integrated a Liconic STX 40 incubator. The direct integration was accomplished by positioning the STX 40 incubator equipped with a Liconic slide station, to the right of the Sciclone ALH 3000 on the same side as the Sciclone's gripper. This allowed the Sciclone's gripper to pick up the plate and move it onto the deck. Another option would have been to use a Twister II robot to move the plates from the incubator to the deck, but this increases the equipment cost of the overall system, and is not as efficient.

Materials

- Sciclone ALH 3000 (Caliper Life Sciences)
- STX 40 incubator equipped with slide station (Liconic Instruments)
- 384 well low volume (LV) microplates - P/N 3826 (Corning)
Total Volume: 50 μ L, Minimum Volume: 5 μ L
- Aquest® fluorescent plate reader (Molecular Devices)
- HEK 293 CRE-luc (Panomics RC0007)
A stable cell line obtained by co-transfection of the CRE containing luciferase construct and pHYG, with selection using hygromycin. This cell line has about 60-fold induction of luciferase activity when compared to untreated cells.
- SteadyLite HTS kit (Perkin Elmer™)

Methods and Results

The luciferase assay protocol was as follows:

1. Harvest HEK 293 CRE-luc cells from culture flask using an enzymatic dissociation solution
2. Seed cells at 2,500 cells/well in 10/20* μ L of (IMDM + 10%FBS + 0.1 mg/mL hygromycin)
3. Induce cells with 2.5/5* μ L of 100 μ M Forskolin
4. Allow the cultures to induce overnight in a 37 °C 5% CO₂ humidity controlled incubator
5. Equilibrate plate at RT for 30 min
6. Add 5/20* μ L of Perkin Elmer SteadyLite reagent
7. Read luminescence on an Acquest (Molecular Devices, Inc.)

*Volumes and concentrations for low volume and normal volume assays, respectively.

I Assay Miniaturization

In previous work, the luciferase reporter gene assay using the Steadylite kit was miniaturized to a total volume of 17.5 μ L. Here we compare performance data of the low volume assay and the normal volume assay. The normal volume assay has a total volume of 45 μ L. Figure 1 shows that there is no significant difference in Z' between the normal and low volume assay formats. Figure 2 shows that the low volume assay has a similar, if not higher, signal to background ratio than the normal volume assay. This is likely due to the more concentrated signal in the low volume microplate wells, as the assays were performed with an equal number of cells per well.

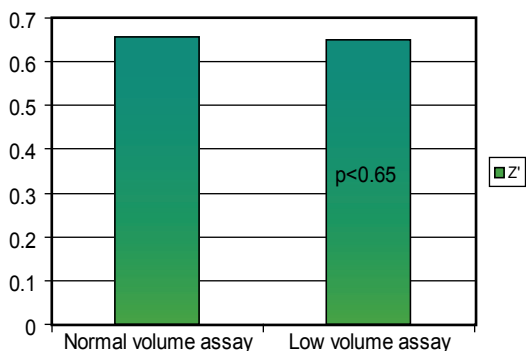


Figure 1. Comparison of Normal and Low volume assay Z' values.

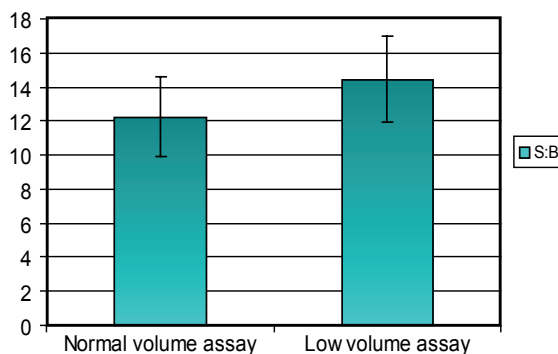


Figure 2. Comparison of Signal to Background values.

II Automation Considerations

Using the Caliper Sciclone ALH 3000 in conjunction with the Liconic STX 40 incubator, we were able to automate the reporter gene assay, and gained the ability to schedule the assay for 40 plates with uninterrupted processing. The automation and scheduling of the assay was programmed using iLINKPro™. iLINKPro allows for system integration, method development, and system operations.

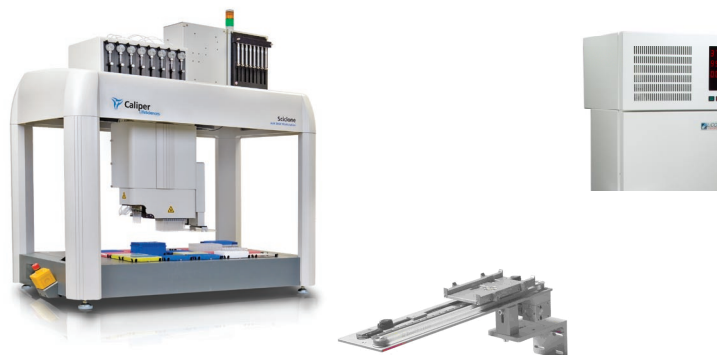


Figure 3 and 4. Sciclone ALH 3000 and Liconic STX 40 with slide.

III Automation Performance

A comparison of the low volume assay when performed by hand and performed by the Sciclone indicated that the Sciclone did not have a significant impact on the signal to background ratio (Figure 5). A comparison of the %CVs, however, shows that the Sciclone has significantly lower %CVs when compared to the assay performed by hand (Figure 6) ($p < 0.001$). This reduction in assay variability resulted in higher Z' scores when performed by the Sciclone (Figure 7) ($p < 0.005$). In addition to reducing the assay variation, the Sciclone was able to process plates faster than by hand.



Figure 5. Comparison of Signal to Background Ratio in Manual vs. Automated Assays.

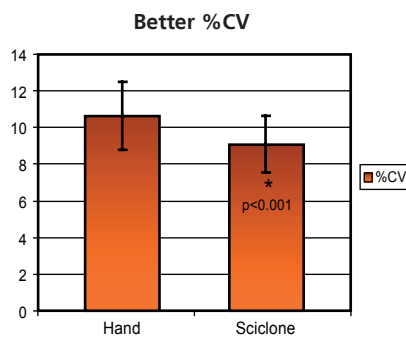


Figure 6. Comparison of Precision for Manual vs. Automated Assays.



Figure 7. Comparison of Z' values for Manual vs. Automated Assays.

IV Cost Reduction

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

Figure 8. Estimate of Cost Reduction due to Miniaturization. Cell culture costs were not included in this estimation, and would also improve savings; the impact would be cell and media dependent.

Conclusions

- The luciferase reporter gene assay can be effectively miniaturized to a low volume 384 well format using a low volume microplate.
- The low volume assay performance is comparable to the normal volume assay in 384 low volume microplates.
- Automation using the Caliper Sciclone and the Liconic STX 40 can lead to reduced variation, a more robust assay, and higher throughput with less manual invention required.
- Miniaturization leads to a 75% reduction in SteadyLite usage.



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