

SOUTHERN RESEARCH

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Abstract

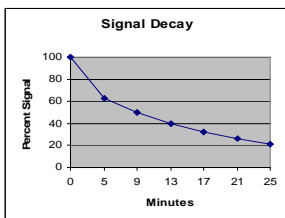
Luciferase has been widely used as a reporter gene in a diverse array of applications. Reagents are available to monitor viability and apoptosis using a luminescent readout. A wide variety of constructs have been made using luciferase to monitor many cellular functions including promoter activity, protein expression, protein interaction and protein fate. The most commonly used luciferase was originally derived from the American firefly (*Photinus pyralis*). Luciferase enzymes have been isolated from other sources but have not enjoyed widespread use. *Renilla* luciferase, isolated from the sea pansy (*Renilla reniformis*) is one of the less widely used luciferases. It has not been popular in HTS applications because the signal decays rapidly. This has necessitated the use of injectors and flash type luminescence to produce consistent signal from sample to sample and plate to plate. For HTS the use of injector equipped readers presents a problem for throughput as this type of read is significantly slower than a standard luminescent read. Injectors also pose mechanical problems in that they can clog and disrupt a screen. In spite of the limitations of *Renilla* luciferase as a reporter it is still used in some applications. The HTS Center at Southern Research Institute recently received such an assay for a 300K compound screen. To meet the throughput requirements of this size screening campaign a method was developed to screen the assay without the use of injector based readers. We present here the methodology used to screen 32,000 compounds a day in what would have otherwise been a very difficult assay to execute.

Evaluation of Stable *Renilla* Luciferase Reagents

Non-injector, HTS friendly endpoint reagents EnduRen (Promega) and Dual-Glo (Promega) (for dual reporter systems using both firefly and *Renilla* reporters) were evaluated for possible use. These reagents provide a longer more stable signal and do not require injectors. Unfortunately, the more stable signal was accompanied by a drop in signal intensity. For assays such as this one with low *Renilla* expression levels, these reagents did not provide robust enough metrics for HTS based on Z values.

Read date	Z Value	
	EnduRen	Dual-Glo
3/14/2007	-0.09	
3/22/2007	0.37	
3/27/2007	0.04	
4/12/2007	0.55	0.42
4/19/2007		0.46

Signal Stability of *Renilla* Luciferase Assay System



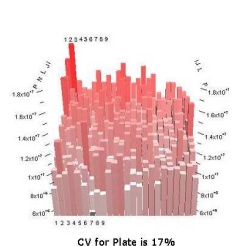
Renilla Luciferase Assay System (Promega) was also evaluated. The signal intensity was much higher than the other endpoints tested, but the signal decayed rapidly. Signal decay of 20-30% was observed over the time required to read a 384-well plate (2 minutes) and there was no signal plateau that would allow a stable two minute read.

HTS Screening of a *Renilla* Based Assay Without the Use of Reader Based Injectors

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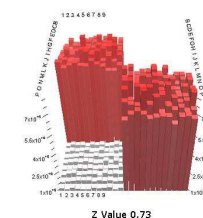
CV Plate Using Standard HTS Equipment



Renilla Luciferase Assay Reagent was dispensed using a Titertek Multidrop which is an eight channel non-contact dispenser. Each tip dispenses to two rows, starting in column 1 and dispensing to columns 1-24, then shifting one row and dispensing to columns 24-1 on the return. This is a common dispense pattern for this type of instrument. The plate reader used, a Perkin Elmer Envision, has a single PMT and each well is read individually. The operator can define the order in which the wells are read. The four options are 1) by row where each row is read from column 1-24, 2) by row bi-directionally where the first row is read from column 1-24, row 2 is read from column 24-1, row 3 is read from column 1-24 etc. 3) by column where each column is read from row A to P and 4) by column bi-directionally which alternates reading down the column and up the next column in a manner similar to the by row bi-directionally. By combining the eight channel bi-directional reagent dispense and the by row bi-directional read, the add to read time for each well was different. Combined with the rapid signal decay observed for this reagent, the CV for the plate was 17% and even though the signal window was good, the high variability produced poor metrics.

Z Plate Using a BioRAPTR

A new strategy was devised to produce a fixed add-to-read time for each well of a microtiter plate. A Beckman BioRAPTR was used to dispense *Renilla* Luciferase Assay Reagent to a plate, matching the by row bi-directional pattern used by the reader. Both the dispense and read required 120 seconds insuring that the add-to-read time for each well was consistent for the plate. Using this strategy, CVs for cells were 6% and for compound were 15%. Combined with a large signal window, this produced Z values consistently above 0.7.



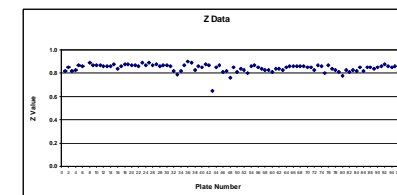
Comparison of *Renilla* Luciferase Reagents

Read date	Z Value		
	EnduRen	Dual-Glo	<i>Renilla</i>
3/14/2007	-0.09		
3/22/2007	0.37		
3/27/2007	0.04		
4/12/2007	0.55	0.42	
4/19/2007		0.46	
5/2/2007			0.71
5/11/2007			0.68
5/18/2007			0.77
5/25/2007			0.74

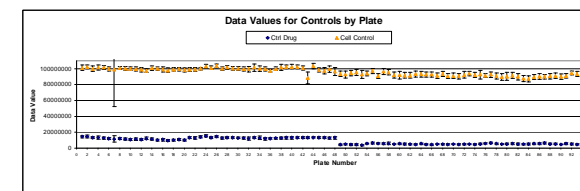
Z values for the *Renilla* Luciferase Assay System, using this strategy, were far better than those produced with the other endpoint reagents. Additional validation was done using this reagent.

Z Values for Validation Screen

As a final step in assay validation, a small library of compounds is screened in duplicate on different days. Z values were generally above 0.8 and raw data values were consistent from run to run and plate to plate.



Data Values for Validation Screen



15,000 compounds in forty-eight 384-well microtiter plates were screened in duplicate as the final step in assay validation. Raw data values were consistent from plate to plate and from day to day.

Conclusions

The problems encountered when trying to use an end point reagent with a rapid rate of decay were overcome by matching dispense pattern and speed to plate reader pattern and speed. This allowed the add-to-read time for each well of a microtiter plate to be maintained for production of consistent and high quality data. The use of this strategy allowed the efficient screening of an assay that would have been difficult to impossible using other equipment, reagents or methods. The application of this strategy extends beyond this specific reagent and can be used when signal stability limits the high throughput application of a readout.

Acknowledgements

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