Optimizing Performance of the Transcreener™ ADP Assay for Commonly Used Multiwell Readers

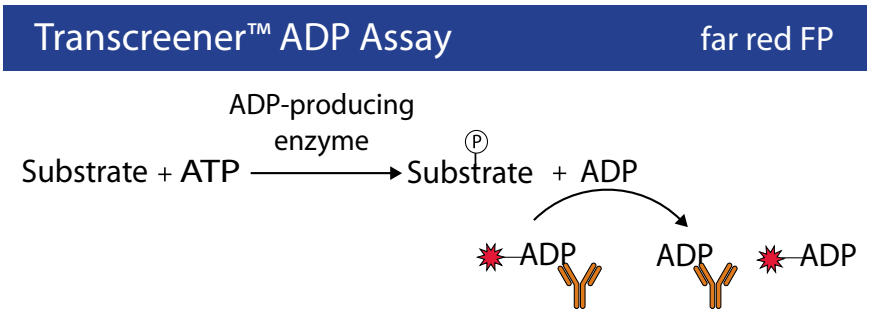
Brad Larson*, Karen Kleman-Leyer, Robert G. Lowery
BellBrook Labs, Madison, Wisconsin, USA 53711

Introduction

There are a number of factors that are driving today's high-throughput screeners to run reactions at low levels of substrate conversion (≤10%). These include the desire for initial velocity kinetics, the need to conserve acceptor substrate, and the need to limit enzyme usage. BellBrook Labs wanted to explore how the Transcreener™ ADP Assay (fluorescence polarization method) performed under conditions of low substrate conversion with commonly used plate readers in 384- and 1536-well formats. Initial experiments examined the effect of flash number, detector gain, and g-factor. We found that by optimizing flash number, and leaving detector gain and g-factor at their instrument recommended settings, the standard Transcreener ADP Assay Kit enabled robust detection of ≤10% substrate conversion, with read times of 1.5-2.5 minutes in 384-well format on the BMG PHERAstar and Tecan Safire²™ and Infinite® F500. However, with the Perkin Elmer EnVision™, read times in excess of 6 minutes were required to meet our internal performance specifications. Additional testing with the EnVision™ was able to show, however, that by increasing the detector gains, we could meet internal specifications of Z′ ≥0.6 at 10% conversion of 10 µM ATP, with a read time of 3 minutes. Further experiments performed on all readers found that we could eliminate the need for longer read times by increasing the concentration of Alexa633 Tracer. This also showed that increased tracer concentration extends the ability to screen at substrate conversion levels well below 10% in all readers, and enhances performance in 1536 well format. For instance, we were able to achieve Z′ greater than 0.5 at ATP conversion levels as low as 2% in 384-well format, and as low as 4% in 1536-well format with acceptable read times. In summary, we have identified conditions for use of the Transcreener ADP Assay that have decreased standard deviations by up to 35% with four of the most commonly used readers in the field today. The combination of BellBrook Labs' Transcreener ADP Assay with high-quality plate reading instrumentation, allows for greater confidence in screening data, decreased time required to complete a screening campaign, and increased productivity for today's high-throughput screening lab.

Figure 1.

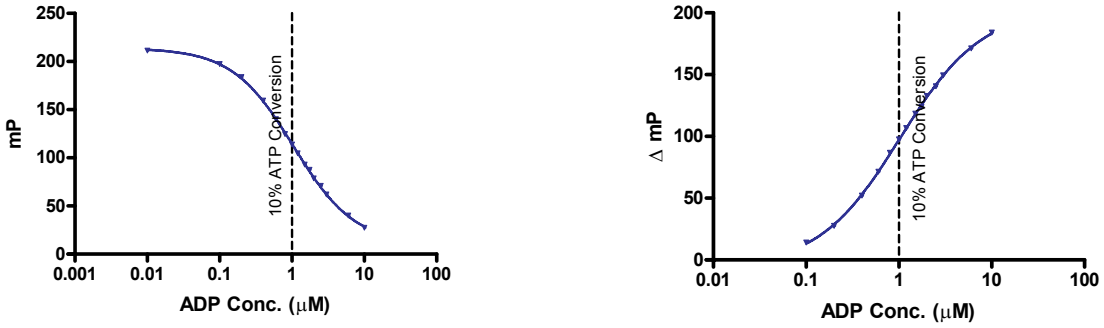
Transcreener™ ADP Assay Principle



The Transcreener ADP Assay is a simple two-part, endpoint assay. 10 µl of the Transcreener ADP Detection Mixture, comprised of an ADP Alexa633 Tracer bound to an anti-ADP Antibody, is added to an equal volume of enzyme reaction mix. ADP, the invariant product generated during the enzyme reaction, displaces the tracer. The displaced tracer freely rotates leading to a decrease in fluorescence polarization. Therefore, ADP production is proportional to a decrease in polarization values.

Figure 2.

10 µM ATP/ADP Standard Curve



Raw polarization (A.) and ΔmP (B.) data shown for 15-point standard curve. The dotted line demonstrates that there is a large assay.

Table 1.
Instrument Summary

Instrument	Excitation	Emission	Dichroic	Flash Number	Read Time
EnVision™	Optimized Cy5 FP Dual Emission Label (Cat#2100-8390)			150	3:01
PHERAstar	Transcreener FP Application Specific Module (Cat#G3M-FP-Trans)			100	3:29
Safire²™	635nm	670/20nm	NA	10	2:28
Infinite® F500	610/20nm	670/25nm	630nm	5	3:03

Evaluations of the Transcreener ADP Assay FP Method were performed on four plate readers commonly found in the field today. Table 1 shows the filter/monochromator and dichroic settings used with each instrument, and also exhibits how instrument specific flash numbers and times vary between vendors.

Table 2.
Effect of Flash Number on Read Time and Robustness of Assay

Dual-Detection Instrumentation						
EnVision™						
Flashes	**100**	**200**	**400**	**500**		
Read Time (Minutes)	**2:17**	**3:43**	**7:00**	**7:55**		
10% ATP Conversion ΔmP	98	118	101	113		
10% ATP Conversion Std. Dev.	9	9	6	6		
10% ATP Conversion Z′-Factor	0.49	0.54	0.61	0.74		
PHERAstar						
Flashes	**30**	**50**	**75**	**100**	**150**	**200**
Read Time (Minutes)	**1:55**	**2:22**	**2:56**	**3:29**	**4:36**	**5:44**
10% ATP Conversion ΔmP	100	102	104	104	105	110
10% ATP Conversion Std. Dev.	7	6	6	5	5	5
10% ATP Conversion Z′-Factor	0.60	0.64	0.65	0.71	0.76	0.79
Single-Detection Instrumentation						
Safire²™						
Flashes	**3**	**5**	**10**	**25**	**50**	
Read Time (Minutes)	**1:22**	**1:35**	**2:28**	**4:37**	**8:25**	
10% ATP Conversion ΔmP	102	102	98	98	97	
10% ATP Conversion Std. Dev.	3	2	3	2	3	
10% ATP Conversion Z′-Factor	0.83	0.87	0.86	0.87	0.86	
Infinite® F500						
Flashes	**3**	**5**	**10**	**20**		
Read Time (Minutes)	**2:22**	**3:03**	**4:41**	**7:20**		
10% ATP Conversion ΔmP	108	109	108	109		
10% ATP Conversion Std. Dev.	5	5	5	5		
10% ATP Conversion Z′-Factor	0.74	0.78	0.76	0.76		

The effect of flash number on data quality was initially tested for each instrument examined.

Table 3.

Effect of Detector Gains on EnVision™ Data

EnVision™: Detector Gain Setting of 655/650				
Flashes	**100**	**200**	**400**	**500**
Read Time (Minutes)	**2:17**	**3:43**	**7:00**	**7:55**
10% ATP Conversion ΔmP	98	118	101	113
10% ATP Conversion Std. Dev.	9	9	6	6
10% ATP Conversion Z′-Factor	0.49	0.54	0.61	0.74
EnVision™: Detector Gain Setting of 800/800				
Flashes	**100**	**150**	**200**	**300**
Read Time (Minutes)	**2:17**	**3:01**	**3:43**	**5:09**
10% ATP Conversion ΔmP	94	95	100	96
10% ATP Conversion Std. Dev.	9	7	7	7
10% ATP Conversion Z′-Factor	0.49	0.60	0.61	0.60

Low counts per flash, approximately 3000, was determined to be the cause of the initial EnVision™ data and read times. Perkin Elmer identified a process where users could increase the detector gain settings of their instrument past normal limits. Experiments were performed to compare data with the new detector gain settings (800/800), to previously generated data, using the standard curve plate setup previously described. The new gain settings increased the counts per flash by a factor of 2, and significantly decreased the read time required for acceptable Z′ values.

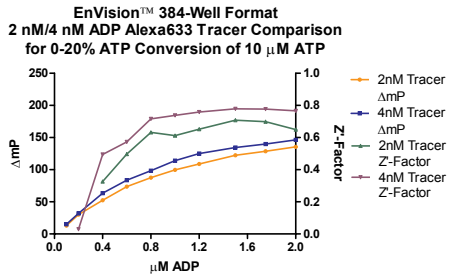
Table 4 and Figure 3.

Effect of Increasing Tracer Concentration from 2 to 4 nM on Read Time and Z′ Values

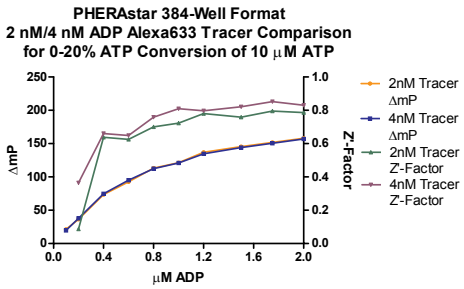
Dual-Detection Instrumentation					
EnVision™					
Flashes	**75**	**100**	**150**	**200**	
Read Time (Minutes)	**1:57**	**2:17**	**3:01**	**3:43**	
10% ATP Conversion ΔmP	112	115	114	113	
10% ATP Conversion Std. Dev.	7	7	5	5	
10% ATP Conversion Z′-Factor	0.60	0.63	0.74	0.76	
PHERAstar					
Flashes	**15**	**30**	**50**	**75**	**100**
Read Time (Minutes)	**1:35**	**1:55**	**2:22**	**2:56**	**3:29**
10% ATP Conversion ΔmP	106	106	117	120	121
10% ATP Conversion Std. Dev.	7	6	6	5	3
10% ATP Conversion Z′-Factor	0.60	0.72	0.78	0.79	0.81
Single-Detection Instrumentation					
Safire²™					
Flashes	**1**	**2**	**3**	**5**	**10**
Read Time (Minutes)	**1:01**	**1:10**	**1:22**	**1:35**	**2:28**
10% ATP Conversion ΔmP	104	102	104	102	109
10% ATP Conversion Std. Dev.	5	3	3	3	2
10% ATP Conversion Z′-Factor	0.73	0.82	0.83	0.86	0.89
Infinite® F500					
Flashes	**1**	**2**	**3**	**5**	
Read Time (Minutes)	**1:44**	**2:03**	**2:22**	**3:03**	
10% ATP Conversion ΔmP	107	108	108	111	
10% ATP Conversion Std. Dev.	3	4	3	4	
10% ATP Conversion Z′-Factor	0.77	0.76	0.81	0.80	

The performance enhancement realized by optimizing gain with the EnVision™ prompted us to examine the effect of increasing tracer concentration. The Transcreener ADP Detection Kit contains 2 nM ADP Alexa633 Tracer. Experiments using the same 15-point standard curve plate set-up were run on each of the four instruments tested to compare the data seen using 2 nM and 4 nM ADP Alexa633 Tracer. Flash number was once again examined to determine the affect of 4 nM tracer on data quality, with equivalent read times.

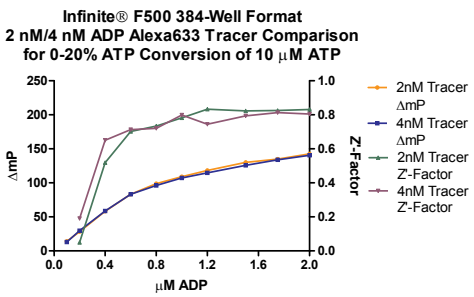
EnVision™



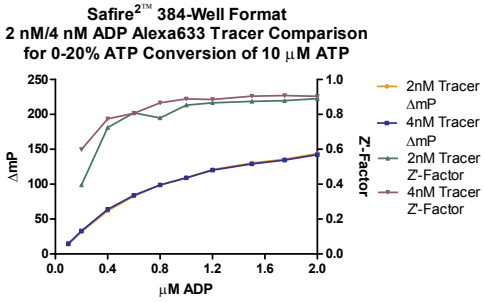
PHERAstar



Infinite® F500



Safire²™



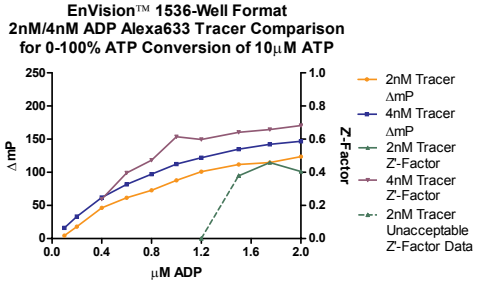
Graphs showing Z' and ΔmP at [ADP] ranging from 2.0-0.1 µM for 2 nM and 4 nM ADP Alexa633 Tracer Detection Systems, in 384-well format. Data generated using 150 flashes with the EnVision™; 100 flashes with the PHERAstar; 10 flashes with the Safire²™; and 5 flashes with the F500.

Figure 4.

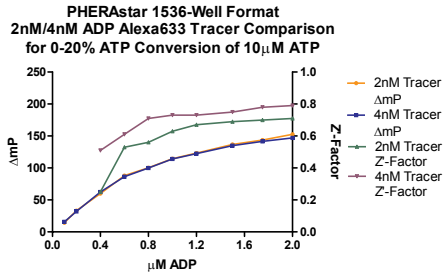
Effect of Increasing Tracer Concentration from 2 to 4 nM on Z'Values in 1536-Well Format

Due to the fact that miniaturized assays use smaller total volumes of enzyme reaction mixture and ADP Detection System, total signal from each well can also decrease. This can then affect the sensitivity of the assay. Because of this, experiments were also run in 1536-well format to compare the effects of 2 nM to 4 nM ADP Alexa633 Tracer Detection Systems.

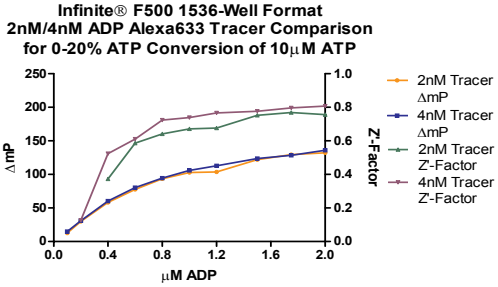
EnVision™



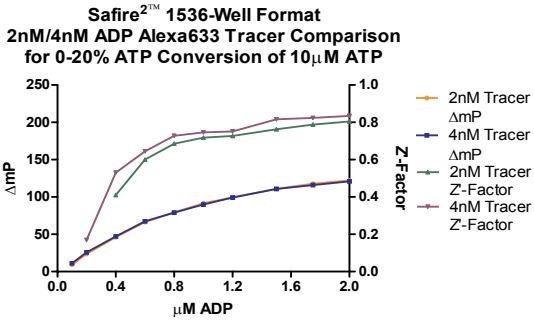
PHERAstar



Infinite® F500



Safire²™



Graphs showing Z' and ΔmP at [ADP] ranging from 2.0-0.1 µM for 2 nM and 4 nM ADP Alexa633 Tracer Detection Systems, in 384-well format. Data generated using 225 flashes with the EnVision™; 100 flashes with the PHERAstar; 10 flashes with the Safire²™ and 10 flashes with the F500.

Materials and Methods

Instrumentation, Plates, and Reader Settings: Fluorescence polarization measurements were performed on the Perkin Elmer EnVision™ 2103, BMG Labtech PHERAstar, Tecan Safire^{2}™, and Tecan Infinite® F500. Assays were performed in Corning 3676 (384-well format) and 3728 (1536-well format) black polystyrene NBS™ microplates. Flash number was varied, while g-factor and gain were kept constant after initial optimization.

Standard Curve: 15-point ATP/ADP standard curves were set up containing decreasing concentrations of ATP and proportionally, increasing concentrations of ADP; the adenine concentration remained constant. The starting concentration of ATP was 10 µM. 24 replicates of each point in the standard curve were manually dispensed (20 µL/well) in 384-well format, and 36 replicates were robotically dispensed (5 µL/well), using a CyBi® –Well 96-Channel Pipettor from CyBio.

Calculations: Z'-Factor, Std. Dev., and mP were generated for each % ATP conversion in the standard curve with each read. Data shown illustrates results at 10% ATP conversion (9 µM ATP/1 µM ADP). mP was calculated by subtracting the mP values at each % ATP conversion level from the mP value at 0% ATP conversion.

Conclusions

- Using vendor recommended settings for the BMG PHERAstar, Tecan Infinite® F500 and Safire^{2}™, in the standard Transcreener™ Assay conditions (2 nM ADP Alexa633 Tracer), enables detection of 10% conversion of 10 µM ATP with Z' > 0.7 in 3.5 minutes or less.
- The data quality obtained with the Perkin Elmer EnVision™ was significantly improved by optimizing the gain settings and by increasing ADP Alexa633 Tracer concentration from 2 to 4 nM.
- Using 4 nM ADP Alexa633 Tracer Detection System may decrease read times and thereby increase the throughput of the screening lab.
- Using 4 nM ADP Alexa633 Tracer Detection System may increase assay sensitivity allowing reactions to be run at lower levels of substrate conversion, thereby decreasing the consumption of valuable substrate and enzyme.
- The Transcreener™ ADP Assay FP Method gave Z' values > 0.7 in 1536-well format using the 2 nM ADP Alexa633 Tracer Detection System on the BMG PHERAstar, and Tecan Infinite® F500 and Safire^{2}™. Use of 4 nM Tracer also improved data quality to varying degrees for all instruments in 1536-well format.

Acknowledgements

BellBrook Labs would like to thank Perkin Elmer, BMG Labtech, and Tecan for allowing us to use their instruments in-house, as well as providing technical assistance during this evaluation.

BellBrook Labs 5500 Nobel Drive, Suite 250, Madison, WI 53711 866.313.7881 or 608.443.2400

www.BellBrookLabs.com