BellBrook Labs

Optimizing Performance of the Transcreener® ADP² FI and GDP FI Assay for the PerkinElmer EnVision® HTS Multilabel Plate Reader

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PerkinElmer instruments

Application Note

In this guide:

Setting recommendations using the PerkinElmer EnVision® for the FI Assays

Introduction

Transcreener® is a universal, high throughput biochemical assay based on detection of nucleotides, which are formed by thousands of cellular enzymes — many of which catalyze the covalent regulatory reactions that are central to cell signaling and are high value targets in drug discovery. The advantages of the Transcreener® HTS Assay Platform over existing assay methods include the following:

Universality
The detection of invariant nucleotide reaction product means that a single set of detection reagents can be used for all of the enzymes in a family and all acceptor substrates.

Far Red Fluorescence Intensity Detection
Use of far red shifted dyes with a simple relative fluorescence output greatly reduces interference and particulate based light scattering from fluorescent compounds.

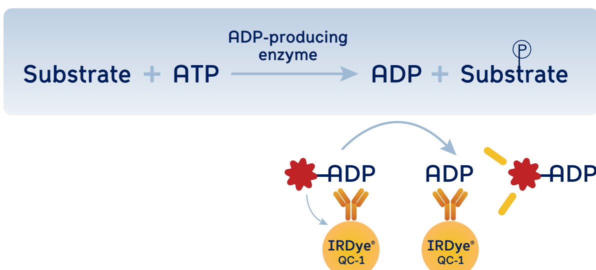
Sensitivity
High affinity antibodies enable robust detection of low levels of substrate conversion (<10%) with less enzyme than other methods.

A critical factor in realizing the numerous advantages of the Transcreener HTS assays is the correct setup of the microplate reader. Proper selection of filters, dichroics, monochromater settings, and read times impact an instrument's sensitivity with any given assay. **Note that the settings are the same for ADP² and GDP FI assay but we have specifically demonstrated the performance using ADP/ATP as the experimental nucleotide.** In this application note we determine the impact of key instrument parameters on Transcreener assay performance. Using this information, the researcher can select the instrument set-up most appropriate for their specific application.

Assay Principle

The Transcreener ADP² FI assay is a simple one-step homogenous detection assay. The Transcreener ADP Detection Mixture comprises an ADP Alexa594 Tracer bound to the ADP Antibody-IRDye® QC-1 quencher conjugate (quencher licensed from LI-COR®). ADP produced by the target enzyme displaces the tracer from the Ab-quencher conjugate which causes an increase in fluorescence. The use of a red tracer minimizes interference from fluorescent compounds and light scattering.

Transcreener® ADP² FI Assay Principle



Performance Criteria

- 384-Well Format
- Z′-Factor ≥ 0.7 at 10% conversion of 10 μM ATP

Standard curves were generated using varying concentrations of ATP and ADP to mimic the conversion of ATP to ADP during the course of an enzyme reaction. Instrument settings were optimized to read the results of the standard curves. Because individual results may vary, optimal setting recommendations should be used as a starting point. Slight modifications may be necessary to optimize results for your particular assay on your instrument.

EnVision® Information

- **Reads Fluorescence Polarization and TR-FRET versions of Transcreener assays**
- **Capable of reading 96, 384, and 1536-well assay plates**
- **Simultaneous dual-detection capabilities**
- **Interchangable filters and dichroic modules**

Materials

Instrument: EnVision® 2100 Multilabel Plate Reader

Microplates: Corning® 384 Well Low Volume Black Round Bottom PS NBS™ Microplate (Product #3676)

Reagent	Kit/Component Catalog #
Transcreener® ADP² FI Assay	3013-1K
ADP Alexa594 Tracer, 800 nM	2073
Stop & Detect Buffer B, 10X	2027
ADP² Antibody-IRDye® QC-1	2074
5mM ADP	2052
5 mM ATP	2053
Buffer Components	
500 mM EGTA	Not Provided
1000 mM HEPES	
500 mM MgCl2	
1% Brij-35	
100% DMSO	

Table 1. Experimental Reagents

Protocol

Standard protocol consists of adding 10 µL of ADP Detection Mixture to 10 µL of the ATP/ADP Standard Mixture in a 384-well plate. The plate was then covered, shaken to mix the reagents, and incubated at room temperature for 60 minutes.

ATP/ADP Mixture

The ATP/ADP mixture consists of 4 mM $MgCl_2$, 2 mM EGTA, 50 mM HEPES, pH 7.5, 1% DMSO, 0.01% Brij-35, and ATP/ADP combined to a constant adenine concentration of 10 µM.

ADP Detection Mixture

The ADP Detection Mixture consists of 1X Stop & Detect Buffer B, 8 nM ADP Alexa594 Tracer, and 10 µg/mL of ADP² Antibody-IRDye® QC-1.

Free Tracer

The Free Tracer consists of 1X Stop & Detect Buffer B, and 8 nM ADP Alexa594 Tracer.

Buffer Blank

The Buffer Blank consists of 1X Stop & Detect Buffer B, and 10 µg/mL ADP² Antibody-IRDye® QC-1.

Final Concentrations in 20 uL Reaction Volume

2 mM $MgCl_2$, 1 mM EGTA, 25 mM HEPES (pH 7.5), 0.5% DMSO, 0.005% Brij-35, ATP/ADP combined to a constant adenine concentration of 5 µM, 0.5X Stop & Detect Buffer B (25 mM HEPES, pH 7.5, 200 mM NaCl, 10 mM EDTA, and 0.01% Brij-35), 4 nM ADP Alexa594 Tracer, and 5 µg/mL of ADP² Antibody-IRDye® QC-1.

Standard Curve Preparation

15-point ATP/ADP standard curves were generated to test the EnVision® Multilabel Plate Reader. ATP/ADP mixtures were created at various concentrations of ATP and ADP listed in Table 2. Final concentration of the buffer components are listed above. Each point on the curve mimics a different substrate conversion level in an enzyme reaction (n=24). 10 µL of each ATP/ADP combination was dispensed across an entire row of a 384-well plate. 10 µL of the buffer was also dispensed to row P of the plate. 10 µL of the prepared ADP Detection Mixture was then dispensed to rows A-O of the assay plate. Finally, in place of the ADP Detection Mixture, 10 µL of free tracer was dispensed to wells P1-P12, and 10 µL of buffer blank was dispensed to wells P13-P24.

Substrate Conversion Levels (%)	ATP, µM	ADP, µM
0	10	0
0.1	9.99	0.01
0.25	9.975	0.025
0.5	9.95	0.05
1	9.90	0.1
2.5	9.75	0.25
5	9.5	0.5
7.5	9.25	0.75
10	9.0	1.0
15	8.5	1.5
20	8	2
30	7	3
50	5	5
70	3	7
100	0	10

Table 2. Standard Curve ATP/ADP Concentrations

Instrument Set-up and Filter Information

Ex. Filter/ Em. Filter		Perkin Elmer Catalog #
Excitation Filter	545/7 nm	2100-5070
Emission Filters	635/15 nm	2100-5590
Mirror	Texas Red FP D595 single mirror	2100-4190

Table 3. Instrument Optics

The following steps were taken to prepare the EnVision to correctly read the Transcreener® ADP² FI Assay:

1. A label was created by replicating an existing label, after the installation of the filters and mirror listed in Table 3.
2. The correct filters and mirror that were installed in the instrument were associated with the new label, in the "General" tab.
3. A new protocol was created using the same replication process mentioned above, and the label and plate to be used were associated with the protocol.
4. The label to be used to read the plate was optimized using the Label Optimization Wizard. The correct protocol was chosen, and then "Plate Dimension", "Measurement Height", and "Detector Gains" were chosen to be optimized.
5. Following completion of the Wizard, the measurement height was recorded from the "Optimization" tab for the label. The optimization was then deleted. The measurement height information was then recorded in the "General" tab for that label.
6. The Label Optimization Wizard was run a second time, with only "Plate Dimension" being chosen for optimization. This once again sends the EnVision® the correct plate dimensions for the test plate, while allowing the detector gains to be increased above the recommended settings of the reader.

Optimized Measurement Settings	
Detector Gain 1	750
Detector Gain 2	0
Measurement Height	11.8
Excitation Light (%)	100
Flash Number	variable

Table 4. Instrument Settings

Fluorescence intensity measurements were performed using the settings listed in Table 4. The Measurement Height, and Excitation Light (%) were optimized prior to reading the plate, using the procedure previously described. The number of flashes per well were manually adjusted in the appropriate label. Flash number was varied to determine the range of read times that would meet the criteria of the instrument validation program.

Calculations

Z′-Factor Calculation

$$Z' = 1 - [(3*SD_{X\%\ conv} + 3*SD_{0\%\ conv}) / |(RFU_{X\%\ conv} - RFU_{0\%\ conv})|]$$

While an assay yielding a Z′ ≥ 0.5 is considered a high quality assay, those producing Z′ values ≥ 0.7 give the user a greater confidence level.

Results

Assay plates containing the 15-point standard curve were read on the EnVision Multilabel Plate Reader (Figure 1). As the ratio of ADP:ATP increases the proportion of bound tracer vs. free tracer decreases resulting in an overall increase in RFU values.

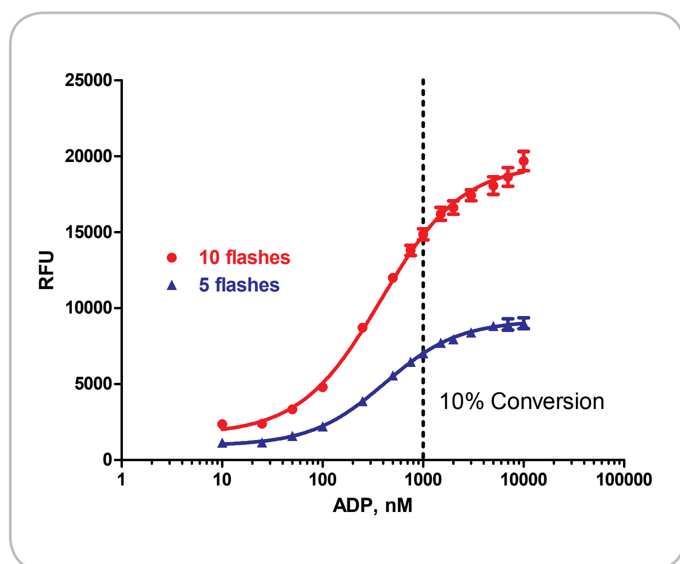


Figure 1. 10 µM ATP/ADP Standard Curve. 10% ATP Conversion represents 9 µM ATP/1 µM ADP concentration level.

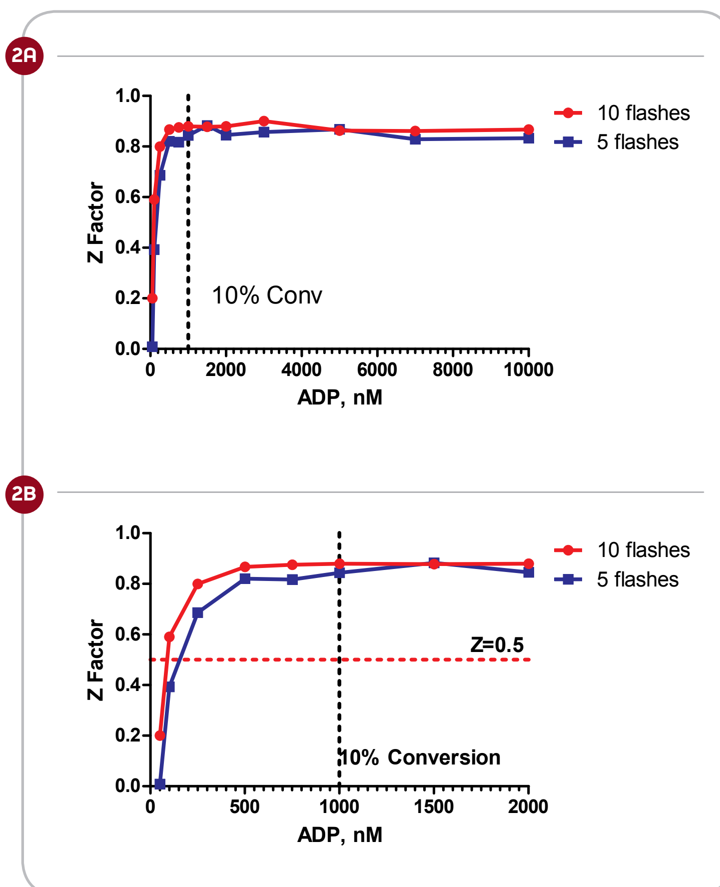


Figure 2. A) Z′ observed in a standard curve mimicking conversion of 10 µM ATP to ADP. B) A zoom in of the 0-2 µM ADP section of the standard curve. Z′ validation minimal qualification shown by red dashed line. 10% ATP conversion validation point shown by black dotted line. Reader set at 10 flashes or 5 flashes.

A Z′ > 0.7 is achieved at 1.0 µM ADP (10% ATP conversion) in a read time of 4:08 (Figure 2A). The Z′ value falls below 0.5 at 0.5% substrate conversion. Figure 2B highlights data that is generated in the initial velocity range of the reaction. Validation criteria are met by the PerkinElmer EnVision® using a reader setting of 5 flashes.

Assay Performance at 10% Conversion of 10 µM ATP						
Flashes	1	5	10	20	50	100
Read Time (Minutes)	4:01	4:08	4:11	4:17	4:85	5:69
% CV at 10% ATP Conversion	6.25	3.22	2.4	1.97	1.88	1.65
Z′-Factor at 10% ATP Conversion	0.69	0.84	0.88	0.91	0.92	0.92

Table 5. Assay performance with various instrument settings.

Variable flashes were evaluated to determine the optimal read time generating the highest quality data. As flash number increases, the percent CV of the mean RFU values decreases slightly, resulting in improved Z′ values (Table 5).

Discussion

The data shows that the Transcreener ADP² and GDP FI Assays are sensitive at low percent ATP conversion levels, thereby making it possible to use smaller amounts of enzyme and substrate in an enzyme reaction. Following the Transcreener protocol and setting the instrument to 5 flashes yielded Z′ values ≥ 0.7 in a read time of 4:08 minutes. The data also shows that the Transcreener ADP² FI Assay improves data quality at low percent ATP conversion levels, thereby making it possible to use smaller amounts of enzyme and substrate in an enzyme reaction.

Conclusions

PerkinElmer's EnVision® Multilabel Plate Reader met the performance criteria under the following conditions: Texas Red D595 single mirror with 545/7 excitation and 635/15 emission filter **combined with 5 instrument flashes. These settings yielded a Z′ > 0.7 with a read time of 4:08 minutes.**

Using the optimized instrument setup recommended by PerkinElmer reduces standard error in RFU measurements.

Transcreener® ADP² FI Assay improves data quality using initial rate enzyme reaction kinetics.

Additional Information

Related Products

Product	Catalog #
Transcreener® ADP² FP Assay	3010-1K
Transcreener® ADP² TR-FRET Red Assay	3011-1K
Transcreener® ADP² FI Assay	3013-1K
Transcreener® AMP/GMP Assay	3006-1K
Transcreener® UDP Assay	3007-1K
Transcreener® GDP FP Assay	3009-1K
Transcreener® GDP FI Assay	3014-1K

Ordering Information

Please contact BellBrook Labs for product pricing.
Custom quotes are available for orders of 10,000 wells or more.

Phone orders:
608 • 443 • 2400
866 • 313 • 7881

Fax orders:
608 • 441 • 2967

Email orders:
info@bellbrooklabs.com

Technical Information

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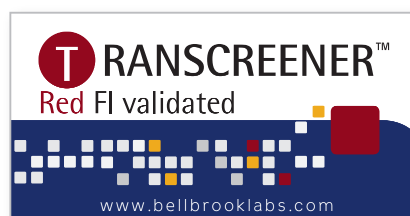
Transcreener Instrument Validation Stickers



Look for the Transcreener Far Red FI-validated sticker on instruments that have successfully met our validation criteria.

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