Application Note

BellBrook Labs

Optimized Settings to Validate the PerkinElmer EnVision®/EnVision® Xcite Microplate Readers with the Transcreener® Fluorescence Intensity Assays.

Meera Kumar[1] and Karen Kleman-Leyer[1]
[1]BellBrook Labs, Madison, WI, USA

This Application Note describes the optimal instrument parameters used to validate the PerkinElmer EnVision®/EnVision® Xcite plate readers with the following assays:
Transcreener® ADP² FI (3013)
Transcreener® GDP FI (3014)

Introduction

Transcreener® HTS is a universal, high throughput biochemical assay platform based on the 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 of great value as targets in drug discovery.

The Transcreener® FI Assays are a single step, competitive immunoassay for direct detection of nucleotides with a red fluorescence intensity (FI) readout. The reagents for all of the assays are a red Tracer bound to a highly-specific monoclonal antibody-quencher conjugate. Nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody-quencher conjugate, resulting in an increase in fluorescence intensity (Figure 1). The use of a red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® FI Assays are designed specifically for HTS with a single addition, mix-and-read format.

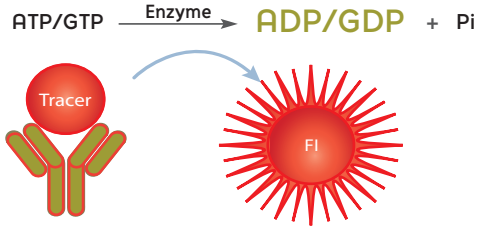

Figure 1. Transcreener® FI Assay Principle

Validation Criteria

A critical factor in realizing the advantages of the Transcreener® HTS assays is the correct setup of the microplate reader used for data readout. Proper selection of filters, dichroics, gain and flashes can impact the instrument`s sensitivity for any given assay. The key instrument parameters for Transcreener® HTS assay performance were identified by running a 10 µM ATP/ADP standard curve (24 replicates), as standard curves of this type mimic enzyme reactions. Starting with 10 µM ATP, ADP was added in increasing amounts and ATP is decreased proportionately, maintaining a total adenine nucleotide concentration of 10 µM. The flash numbers were varied to determine the requirements for a Z' > 0.5. ***In order to validate an instrument for use with the Transcreener® FI Assays, a Z' > 0.7 at 10% conversion of 10 µM ATP was required.***

EnVision®/EnVision® Xcite Information

- Reads Fluorescence Intensity, Fluorescence Polarization and TR-FRET versions of the Transcreener® Assays.
- Capable of reading 96-, 384- and 1536-well plates.
- Simultaneous dual detection capabilities.
- Interchangeable filters and dichroic modules.

PerkinElmer™ instruments.

Instrument Settings

Instrument Wavelength Settings (PerkinElmer Catalog #)	
Excitation Filter	547/7 nm (2100-5070
Emission Filters	635/15 nm (2100-5590
Mirror	Texas Red FP D595 (2100-4190
	Single Mirror
Optimized EnVision® Settings	
Detector Gain 1	750
Detector Gain 2	0
Measurement Height	11.8
Excitation Light (%)	100
Flash Number	Variable

Table 1. Recommended PerkinElmer EnVision® Instrument Settings

Instrument Setup

The Transcreener-specific FI mirrors and filters were installed prior to instrument evaluation. Once those components have been installed, proceed with the following steps to optimize the detector gains and Z-height focus:

1. Create a label by replicating an existing label.
2. Associate the installed filters and mirror with the new label in the "***General***" tab.
3. Create a new protocol by replicating an existing protocol and associate the new label and a plate with the protocol.
4. Add ***20 µL*** of ***Free Tracer (2 nM)*** to the four corners of the plate (in the buffer conditions of your enzyme reaction).
5. Run the ***Label Optimization Wizard*** to optimize the label. Select the appropriate protocol and then select "***Plate Dimension***", "***Measurement Height***", and ***"Detector Gains"*** to proceed with the ***Label Optimization Wizard***.
6. Record the "***Measurement Height***" value from the "***Optimization***" tab. Next, delete the optimization. Then, input the ***Measurement Height*** value into the "***General***" tab for the label.
7. Run the ***Label Optimization Wizard*** again. This time, select only "***Plate Dimension***" for optimization. This allows the instrument to use the correct plate dimensions, while allowing the detector gains to be increased above the reader's recommended settings.

The same measurement settings can be used for subsequent plates as long as the volumes, tracer and concentrations remain the same. A snapshot of the ***FI Transcreener Assay 595 Mirror*** screen is shown in Figure 2.

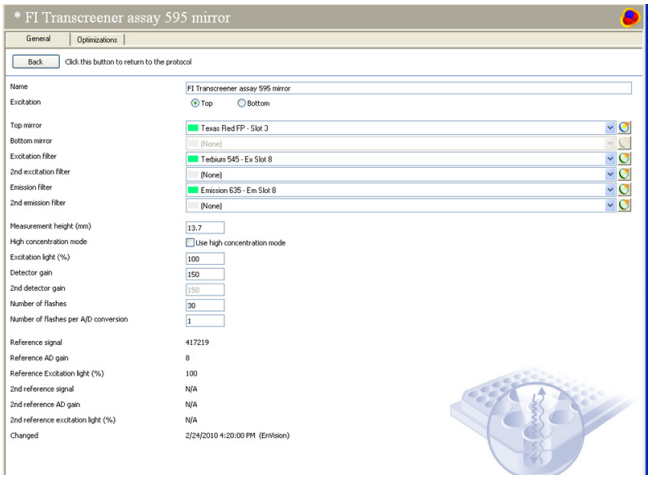

Figure 2. Screen Snapshot of FI Transcreener Assay 595 Mirror Dialog Box

Sample FI Standard Curve

As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall increase in RFU values. Assay plates containing the 15-point standard curve were read on the EnVision® Microplate Reader.

Materials

ATP/ADP Mixture - 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 - 1X Stop & Detect Buffer B, 8 nM ADP Alexa594 Tracer, and 10 µg/mL ADP^2 Antibody-IRDye®QC-1.

Free Tracer - 1X Stop & Detect Buffer B and 8 nM ADP Alexa594 Tracer.

Buffer Blank - 1X Stop & Detect Buffer B and 10 µg/mL ADP^2 Antibody-IRDye®QC-1.

For a detailed procedure on how to prepare a standard curve, please refer to the appropriate Transcreener® Technical Manual (http://www.bellbrooklabs.com/transcreener_hts_assays.html).

Method

1. Dispense 10 µL of each ATP/ADP combination across an entire row of a 384-well plate.
2. Add 10 µL of ADP Detection Mix to those rows.
3. Dispense 10 µL of the 10 µM ATP/0 µM ADP combination into row P.
4. Dispense 10 µL of Free Tracer into wells P1-P12.
5. Dispense 10 µL of Buffer Blank into wells P13-P24.

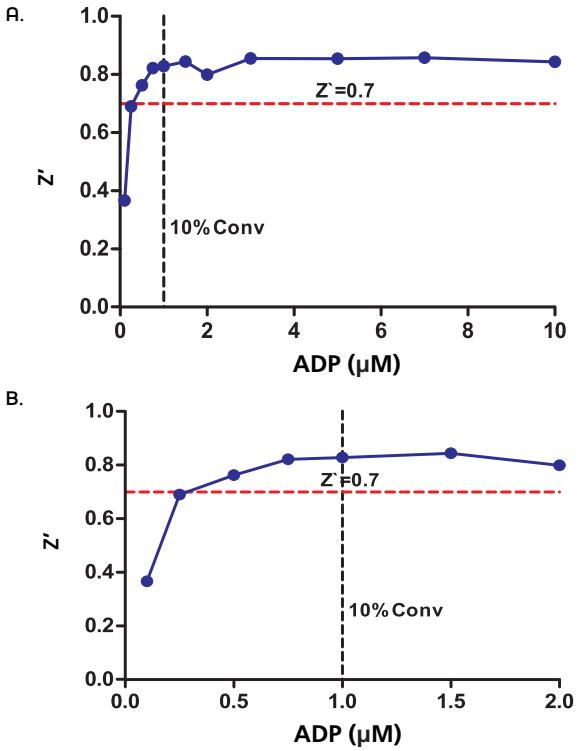

Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set at 10 flashes.

Assay Performance, 10% Conversion 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	2.40	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 2. Assay Performance with Various Instrument Settings

Conclusions

This application note demonstrates the validation of the PerkinElmer EnVision® and EnVision® Xcite microplate readers for use with the Transcreener® FI Assays. By utilizing the optimized instrument settings suggested within this Application Note, Z' values > 0.7 are achievable.

Additional Information

Ordering Information

Please visit www.bellbrooklabs.com or contact BellBrook Labs for pricing for the Transcreener® HTS Assays. Custom quotes are available for bulk orders.

Phone Orders:
608.443.2400
866.3137881

Fax Orders:
608.441.2967

Email Orders:
info@bellbrooklabs.com

Related Products

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

Technical Information

For technical information, please contact:

Meera Kumar, Applications Scientist
Tel: 608.443.2400
Toll-Free: 866.313.7881
Email: meera.kumar@bellbrooklabs.com

References & Notes

Transcreener® HTS Assay Platform is a patented technology of BellBrook Labs.

Transcreener® is a registered trademark of BellBrook Labs.

EnVision® is a registered trademark of PerkinElmer.

AlexaFluor® is a registered trademark of Molecular Probes, Inc (Invitrogen).

IRDye®QC-1 is a registered trademark of LI-COR Biosciences.

BellBrook Labs