

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

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This Application Note describes the optimal instrument parameters used to validate the PerkinElmer EnVision®/EnVision® Xcite plate readers with the following assays:

*Transcreener® ADP² FP (3010) Transcreener® AMP/GMP (3006)
 Transcreener® UDP FP (3007) Transcreener® GDP FP (3009)*

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® FP Assays are a single step, competitive immunoassay for direct detection of nucleotides with a far red fluorescence polarization (FP) readout. The reagents for all of the assays are a far red Tracer bound to a highly-specific monoclonal/polyclonal antibody. Nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody, leading to increased rotational freedom and results in a decrease in polarization (Figure 1). The use of a far red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® FP Assays are designed specifically for HTS with a single addition, mix-and-read format.

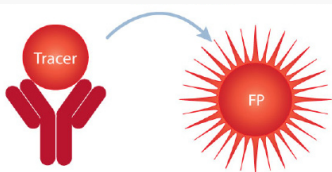
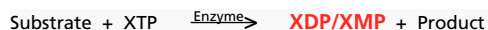


Figure 1. Transcreener® FP Assay Principle

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® FP Assays, $Z' > 0.7$ and a $\Delta mP > 120$ at 10% conversion of 10 μM ATP were required.*

EnVision®/EnVision® Xcite Information

- Multidetector HTS microplate reader with advanced fluorescence polarization capabilities.
- Full modularity in all detection modes covered by two matched pairs of PMT's and optimized assay specific optical modules.
- Simultaneous dual emission (SDE) detection for fastest read times and highest sensitivity.
- Assay flexibility provided by precise temperature control and multimode shaking capabilities.



Instrument Settings

EXC Filter/EMS Filter	PerkinElmer Catalog #	
Excitation Filter	620/40 nm	2100-5760
Emission Filters	688/45 nm (S)	2100-5780
Emission Filters	688/45 nm (P)	2100-5790
Mirror	D658/fp688 Dual Mirror	2100-4260
Package	Optimized Cy5 FP Dual Emission Label	2100-8390

Optimized EnVision® Settings

Detector Gain 1	800
Detector Gain 2	800
Measurement Height	12
Excitation Light (%)	100
Flash Number	Variable
G-Factor	0.62

Table 1. Recommended PerkinElmer EnVision® Instrument Settings

Instrument Setup

The Transcreener-specific FP 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 at 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 "G-Factor" and "Measurement Height" values from the "Optimization" tab. Next, delete the optimization. Then, input the G-Factor and Measurement Height values 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 *Transcreener Label* screen is shown in Figure 2.

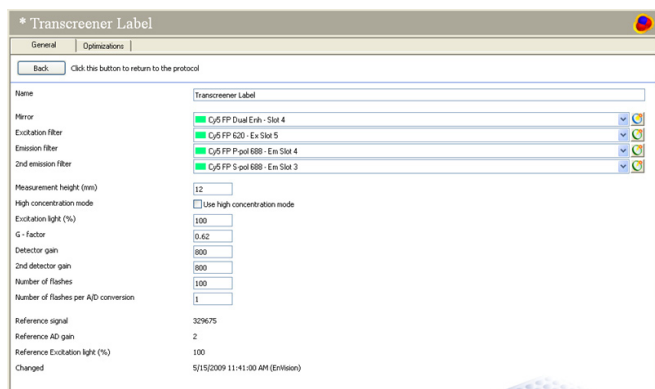


Figure 2. Screen Snapshot of Transcreener Label Dialog Box

Sample FP Standard Curve

As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in mP 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, 4 nM ADP Alexa633 Tracer, and 14.8 $\mu\text{g}/\text{mL}$ ADP² Antibody.

Free Tracer - 1X Stop & Detect Buffer B and 4 nM ADP Alexa633 Tracer.

Buffer Blank - 1X Stop & Detect Buffer B and 14.8 $\mu\text{g}/\text{mL}$ ADP² Antibody.

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.

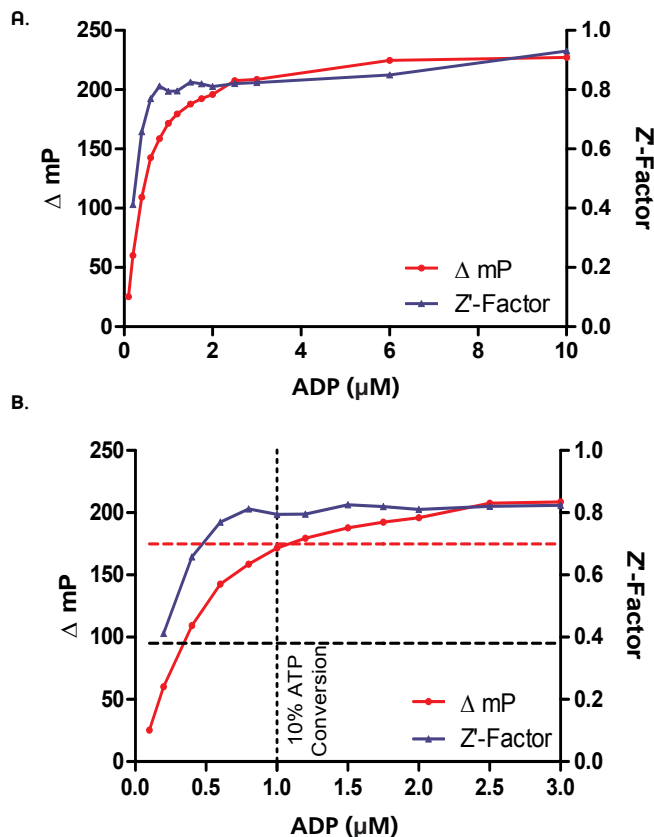


Figure 3. A). Z' and Δ mP values observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-3 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and Δ mP validation minimal qualification data (black dashed line). 10% ATP conversion validation point is shown by the black dotted line. Plate reader set at 30 flashes.

Assay Performance, 10% Conversion 10 μM ATP						
Flashes	30	50	75	100	150	200
Read Time (minutes)	1:20	1:37	2:00	2:17	3:01	3:43
Δ mP at 10% ATP Conversion	172	173	170	170	168	167
Standard Deviation at 10% ATP Conversion	6	5	4	3	4	3
Z'-Factor at 10% ATP Conversion	0.79	0.83	0.86	0.88	0.87	0.89

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® FP Assays. By utilizing the optimized instrument settings suggested within this Application Note, Z' values > 0.7 and $\Delta mP > 120$ 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® ADP ² FP Assay.....	3010-1K
Transcreener® ADP ² FI Assay.....	3013-1K
Transcreener® ADP ² 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

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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).

The Transcreener® product line is the subject of U.S. Patent No. 7,332,278, 7,355,010 and 7,378,505 issued. U.S. Patent Application Nos. 11/353,500, 11/958,515 and 11/958,965, U.S. Divisional Application 12/029,932, and foreign equivalents licensed to BellBrook Labs.

