

Optimized Settings to Validate the PerkinElmer EnVision®/EnVision® Xcite Microplate Readers with the Transcreener® TR-FRET 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 TR-FRET (3011)

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® TR-FRET Assays are a single step, competitive immunoassay for direct detection of nucleotides with a far red time-resolved Förster-resonance-energy-transfer (TR-FRET) readout. The reagents for all of the assays are a far red Tracer bound to a highly-specific monoclonal antibody-Terbium conjugate. Excitation of the Terbium complex in the UV range (ca. 330 nm) results in energy transfer to the Tracer and emission at a higher wavelength (665 nm) after a time delay. Nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody, leading to a decrease in TR-FRET (Figure 1). The use of a red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® TR-FRET Assays are designed specifically for HTS with a single addition, mix-and-read format.

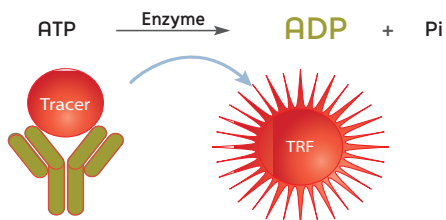


Figure 1. Transcreener® TR-FRET Assay Principle

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® TR-FRET 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.



Instrument Settings

Instrument Wavelength Settings		PerkinElmer Catalog #
Excitation Filter	320/7.5 nm	2100-5060
Emission Filter 1	615/8.5 nm	2100-5090
Emission Filter 2	665/7.5 nm	2100-5110
Mirror	Lance Dual D400/D630	2100-4160

Optimized EnVision® Settings	
Window Time	100 µs
Measurement Height	11.6 mm
Delay	60 µs
Flash Number	Variable
Flash Number (2nd Detector)	20
Time between Flashes	2000 µs
Excitation Light	100%

Table 1. Recommended PerkinElmer EnVision® Instrument Settings

Instrument Setup

The Transcreener-specific TR-FRET mirrors and filters were installed prior to instrument evaluation. Once those components have been installed, proceed with the following steps:

1. Create a label by replicating an existing label (*Lance-TRF* under Wallac protocols).
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", and "Measurement Height" 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 *BellBrook TR-FRET Red* screen is shown in Figure 2.

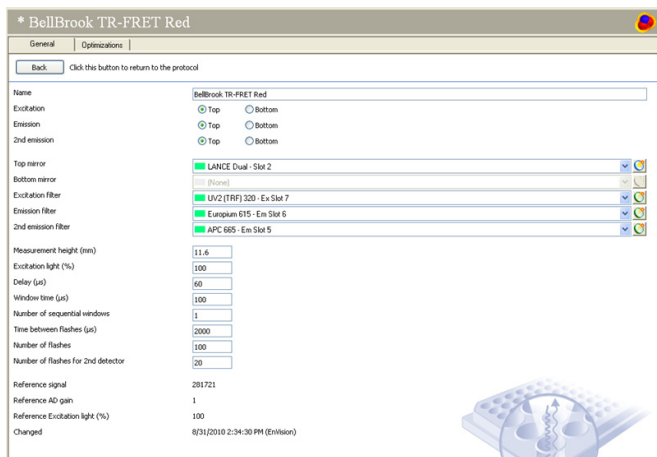


Figure 2. Screen Snapshot of BellBrook TR-FRET Red Dialog Box

Sample TR-FRET Standard Curve

As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in FRET. 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 C, 8 nM ADP² Antibody-Tb, and 27 nM ADP HiLyte647 Tracer.

High FRET Mixture - 8 nM ADP² Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 μM ATP in 1X Stop & Detect Buffer C.

Low FRET Mixture - 8 nM ADP² Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 μM ADP in 1X Stop & Detect Buffer C.

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 the High FRET mixture into wells P1-P12.
5. Dispense 10 μL of the Low FRET mixture into wells P13-P24.

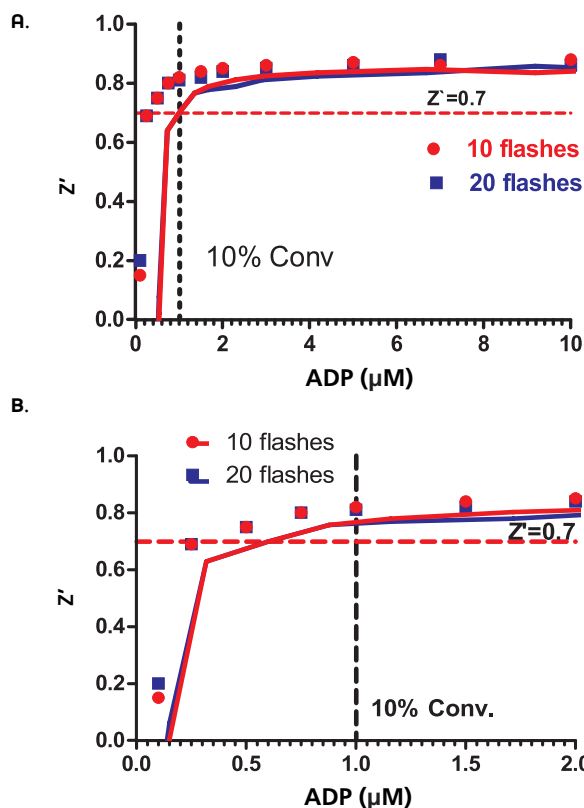


Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-2 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set at 10 or 20 flashes.

Assay Performance, 10% Conversion 10 μM ATP					
Flashes	1	5	10	20	100
Read Time (minutes)	1:27	2:22	4:34	5:06	7:24
% CV at 10% ATP Conversion	8.85	4.91	3.66	3.32	3.16
Z' -Factor at 10% ATP Conversion	0.51	0.65	0.82	0.81	0.84

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® TR-FRET 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® 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.

LanthaScreen® Terbium is a registered trademark of Invitrogen (Life Technologies).

HiLyte Fluor™ is a trademark of Anaspec.

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.

