

Optimized Settings to Validate the Tecan Infinite® F500 HTS Microplate Reader with the Transcreener® Fluorescence Polarization Assays.

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This Application Note describes the optimal instrument parameters used to validate the Tecan Infinite® F500 plate reader 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.

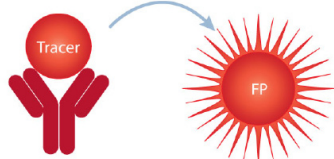


Figure 1. Transcreener® FP Assay Principle

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

Infinite® F500 Information

- **Automated Z-Focus** - Automatically adjust the Z-focus in all top reading modes (Fluorescence Intensity, Fluorescence Polarization, and Luminescence); significantly improving the quality of measurements.
- **Programmable Filter Slide** - Use of separate excitation and emission filter slides with six filter positions each provides a lot of flexibility for running a variety of assays.
- **High Measurement Speed & Robotic Compatibility** - Capable of reading 6- to 384-well plates, including standard, half-area and low volume 384-well plates. Top reading modes can be used with 1536-well microplates.
- **Integrated Injectors** - Optionally equipped with two injectors that are integrated into the plate reader.
- **i-Control™ Software** - Convenient workflow-oriented reader control.




Instrument Settings

Instrument Wavelength Settings

EXC Wavelength and Bandwidth	610/20 nm
EMS Wavelength and Bandwidth	670/25 nm
Mirror	Dichroic 630 (Cy5)

Optimized Infinite® F500 Settings

Gain	Optimal
Integrated Time	20 μs
Settle Time	50 msec
Flash Number	1-20

Table 1. Recommended Tecan Infinite® F500 Instrument Settings

Instrument Setup

1. Open the Tecan i-control™ software, connect to the Infinite® F500 reader, and open a new method file.
2. Select the appropriate labware/plate from the "Plate Definition" dropdown menu, and select the portion of the labware/plate to be read.
3. Select "Fluorescence Polarization" from the "Measurements" menu.
4. Select "610 (10 nm)" from the Excitation dropdown menu and "670" for Emission (25.0 nm for Bandwidth).
5. To allow the instrument to calculate the proper gain based on each well, select "Optimal" for the Gain. If the plate is being read more than once, select "Manual" for the Gain and input the value that the instrument previously calculated.
6. To set the measurement blank, select "Change" from the "Measurement" section and select the wells containing the buffer blank.

The same measurement settings can be used for subsequent plates as long as the volumes, tracer and concentrations remain the same.

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 Tecan Infinite® F500 Microplate Reader.

Materials

ATP/ADP Mixture - 4 mM MgCl₂, 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 μg/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 μg/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.

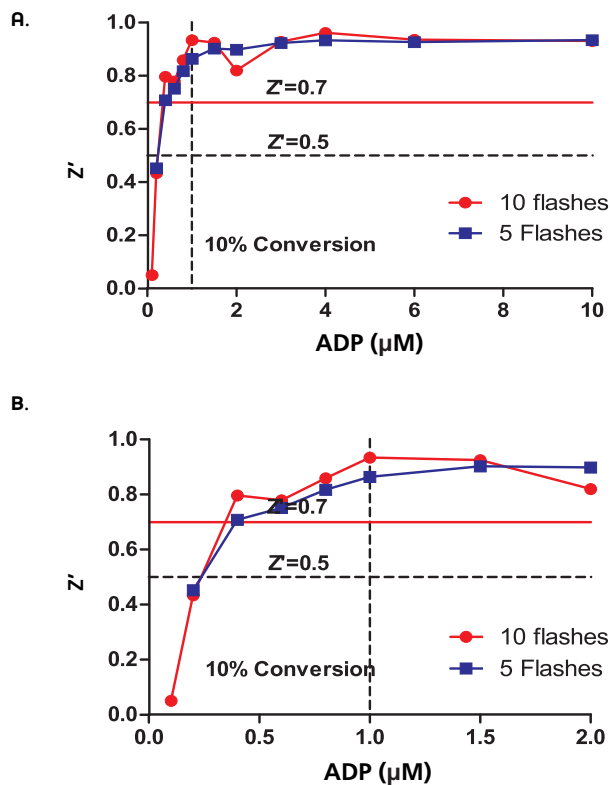


Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-2 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set at 10 or 5 flashes.

Assay Performance, 10% Conversion 10 μM ATP						
Flashes	1	3	5	10	15	20
Read Time (minutes)	1:08	1:20	1:33	2:04	2:38	3:08
ΔmP at 10% ATP Conversion	137	137	136	137	134	135
Z'-Factor at 10% ATP Conversion	0.84	0.89	0.86	0.93	0.89	0.91

Table 2. Assay Performance with Various Instrument Settings

Conclusions

This application note demonstrates the validation of the Tecan Infinite® F500 microplate reader for use with the Transcreener® FP Assays. By utilizing the optimized instrument settings suggested within this Application Note, Z' values > 0.7 and ΔmP > 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.

Infinite® is a registered trademark of Tecan.

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.

