Application Note

BellBrook Labs

Optimized Settings to Validate the Tecan Infinite® F500/F200/Ultra[evolution] HTS Microplate Readers with the Transcreener® Fluorescence Intensity Assays.

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This Application Note describes the optimal instrument parameters used to validate the Tecan Infinite® F500/F200/Ultra[evolution] plate readers with the following assays:
Transcreener® ADP[2] 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.
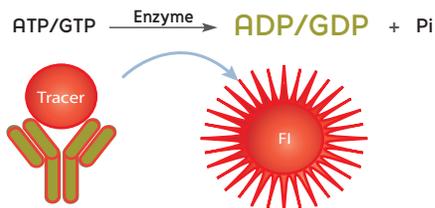

$$\text{ATP/GTP} \xrightarrow{\text{Enzyme}} \text{ADP/GDP} + \text{Pi}$$

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® FP Assays, a Z' > 0.7 at 10% conversion of 10 µM ATP was required.*

Infinite® F500/F200/Ultra[evolution] Info

- **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.

TECAN.

Instrument Settings

Instrument Wavelength Settings

EXC Wavelength and Bandwidth	580/20 nm
EMS Wavelength and Bandwidth	620/20 nm

Optimized Infinite® F500 Settings

Gain	Optimal
Z-Position	Calculate from Well Containing Fluor
Settle Time	0 µs
Flash Number	1-20
Integration Time	40 µs
Mirror (Automatic)	50%

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 Intensity*" from the "*Measurements*" menu.

4. Select *"580 (20) nm"* from the *Excitation* dropdown menu and "*620*" for *Emission (20.0 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 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 Tecan Infinite® F500 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 ADP2 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 ADP2 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.

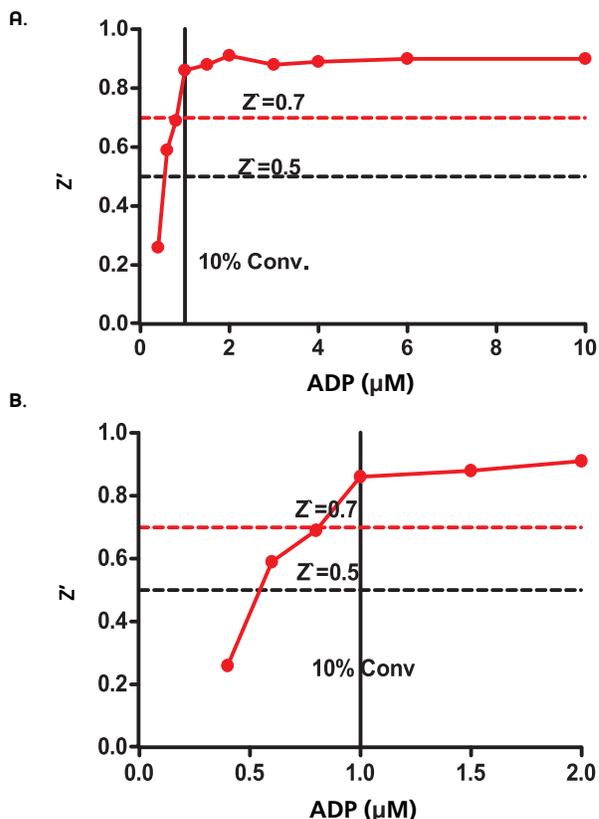5. Dispense 10 µL of Buffer Blank into wells P13-P24.

A.

B.

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.

Conclusions

This application note demonstrates the validation of the Tecan Infinite® F500/F200 and Ultra^evolution 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.

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

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References & Notes

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

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

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