# Application Note

BellBrook Labs

## Optimized Settings to Validate the Tecan Infinite® M1000/M200/Safire² ™* 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® M1000/M200/Safire² ™ 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.

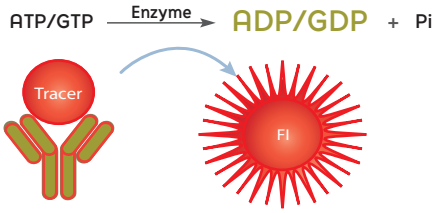


**Figure 1. Transcreener® FI Assay Principle**

## 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.***

## Infinite® M1000/M200/Safire²™ Info

- **Quad-4 Monochromators™** technology.
- **Z-Focusing in Top Reading Modes -** Fluorescence Intensity, Fluorescence Polarization, and Luminescence.
- **User-Selected Frequency Modes (patented) -** Choose between "High Sensitivity" and "High Speed" settings to adjust for individual assay needs.
- **Fast Ratiometric Measurements -** Immediate changes in wavelength in (TR)-FRET based assays and instant change of polarization direction for FP measurements.
- **Free Definition of Plate Formats -** Up to 1,536 wells.
- **Top & Bottom Reading -** Variety of microplate formats in the fluorescence mode.
- **Modular Design -** Allows upgrades to new detection modes at any time if further applications are required.
- **Optional Stacker Module -** Batch processing of up to 50 microplates.
- **i-Control™ Software** - Convenient workflow-oriented reader control.

TECAN

## Instrument Settings

| Instrument Wavelength Settings | |
|---|---|
| EXC Wavelength and Bandwidth | 580/10 nm |
| EMS Wavelength and Bandwidth | 620/10 nm |

| Optimized Infinite® M1000 Settings | |
|---|---|
| Gain | Optimal |
| Z-Position | Calculate from Well containing Fluor |
| Settle Time | 0 µs |
| Flash Number | 1-20 |
| Integration Time | 20 µs |
| Flash Frequency | 100 Hz |

**Table 1. Recommended Tecan Infinite® M1000 Instrument Settings**

* Safire²™ Instrument No Longer Commercially Available.

## Instrument Setup

1. Open the Tecan i-control™ software, connect to the Infinite® M1000 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 (10) nm***" from the ***Excitation*** dropdown menu and "***620***" for ***Emission (10.0 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® M1000 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.

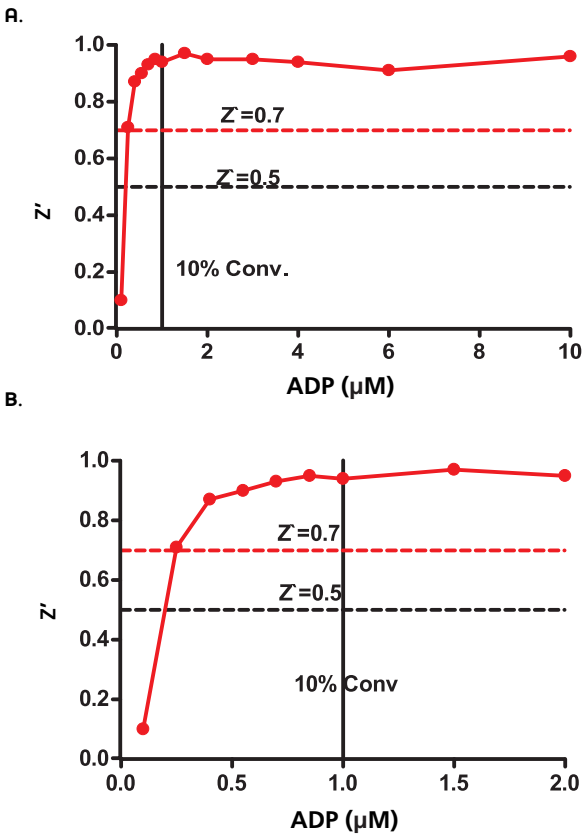


**Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader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

## Conclusions

*This application note demonstrates the validation of the Tecan Infinite® M1000/M200 and Safire²™ 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

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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.

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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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