

## Optimized Settings to Validate the Molecular Devices SpectraMax® M5/M5e & FlexStation® 3 Microplate Readers with the Transcreener® Fluorescence Intensity Assays.

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*This Application Note describes the optimal instrument parameters used to validate the SpectraMax® M5/M5e & FlexStation® 3 plate readers with the following assays:*

*Transcreener® ADP<sup>2</sup> 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.

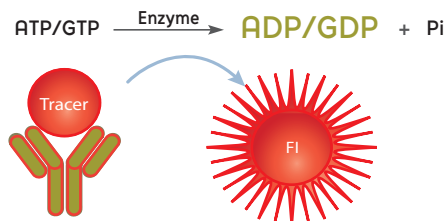


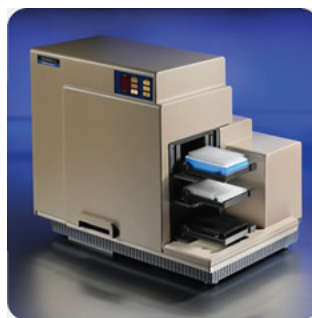
Figure 1. Transcreener® FI Assay Principle

### 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.*

### SpectraMax® M5/M5e/FlexStation® 3

- Uses dual monochromators for variable wavelength selection between 250 nm and 850 nm.
- Patented AutoPMT Optimization System adjusts the fluorescence detector to each sample well's concentration and normalizes the raw data, extending the dynamic range of assays so that low and high signals can be captured from the same plate.
- Supplied with SoftMax® Pro Data Acquisition & Analysis Software.
- Supports 6 to 384 well microplates.



 Molecular Devices

### Instrument Settings

Instrument Wavelength Settings	
EXC Wavelength	575 nm
EMS Wavelength	620 nm
Auto Cutoff	610 nm
Optimized SpectraMax® M5/M5e Settings	
Readings	10-50
PMT	Auto
Auto Calibrate	On

Table 1. Recommended SpectraMax® M5/M5e & FlexStation® 3 Instrument Settings

## Instrument Setup

The SpectraMax® M5/M5e and FlexStation® 3 instruments use SoftMaxPro® Data Acquisition & Analysis Software. Proceed with the following steps to optimize the instrument:

1. Open the SoftwarePro® Data Acquisition & Analysis software, and select "Fluorescence" for the Read Mode.
2. Select "575" for the Excitation, and "620" for the Emission from the Wavelengths menu. Use the default Auto Cutoff of "610 nm".
3. Set Readings to 10 and PMT to Auto from the Sensitivity tab.
4. Set Autocalibrate to "On", select the wells to be read, and click "OK".

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 Settings screen is shown in Figure 2.

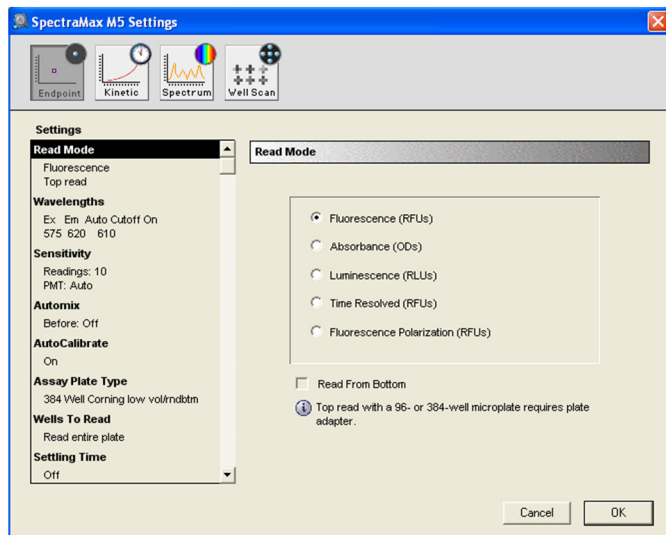


Figure 2. Screen Snapshot of Settings Dialog Box

## 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 Molecular Devices' SpectraMax® M5 and FlexStation® 3 Microplate Reader.

### Materials

**ATP/ADP Mixture** - 4 mM MgCl<sub>2</sub>, 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 ADP<sup>2</sup> 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 ADP<sup>2</sup> 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](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.

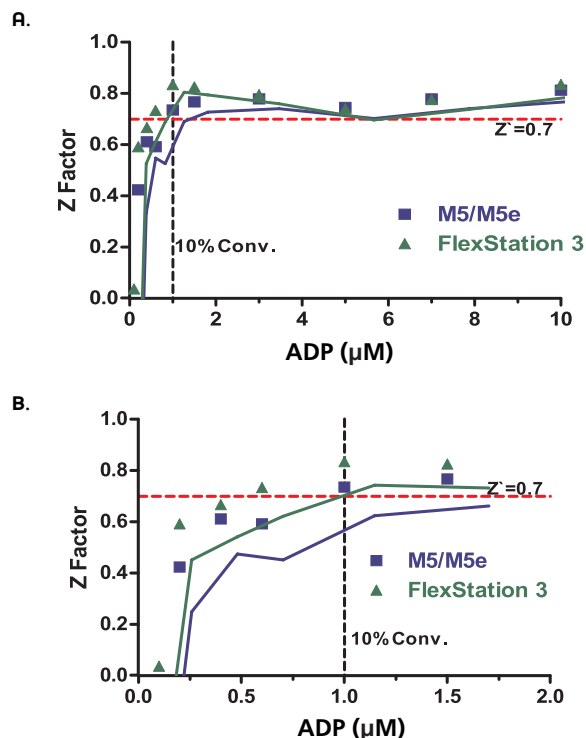


Figure 2. A). Z' values observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-2 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line.) Plate reader set to 50 reads.

Assay Performance, 10% Conversion 10 μM ATP		
Reads	10	50
Z'-Factor at 10% ATP Conversion (M5/M5e)	0.68	0.72
Z'-Factor at 10% ATP Conversion (FlexStation 3)	0.72	0.82

Table 2. Assay Performance with Various Instrument Settings

## Conclusions

This application note demonstrates the validation of the Molecular Devices SpectraMax® M5/M5e & FlexStation® 3 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](http://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](mailto:info@bellbrooklabs.com)

### Related Products

Transcreener® ADP <sup>2</sup> FP Assay.....	3010-1K
Transcreener® ADP <sup>2</sup> FI Assay.....	3013-1K
Transcreener® ADP <sup>2</sup> 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.

SpectraMax® and FlexStation® are registered trademarks of Molecular Devices.

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

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

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.

