Application Note

BellBrook Labs

Optimized Settings to Validate the Molecular Devices SpectraMax® M5/M5e & FlexStation® 3 Microplate Readers with the Transcreener® TR-FRET Assays.

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

Transcreener® ADP² TR-FRET Red Assay (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 immunoassays 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.

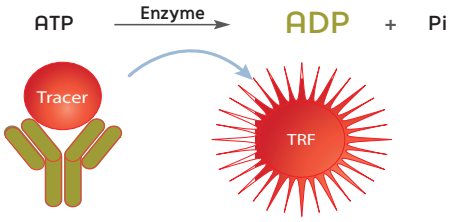

Figure 1. Transcreener® TR-FRET Assay Principle

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.***

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	320 nm
EMS Wavelength	665 nm
EMS Wavelength	620 nm

Optimized SpectraMax® M5/M5e & FlexStation® 3 Settings	
Read Mode	Time Resolved
Delay	50 µsec
Integration	500 µsec
Read Position	Top
Readings/Well	100
PMT	Auto

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

Instrument Setup

The SpectraMax M5/M5e & 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 "***Time Resolved***" for the ***Read Mode***.
2. Set the ***Integration Delay*** to "***50 µs***" and the ***Integration Time*** to "***500 µs***".
3. Enter "***2***" from the ***Wavelengths*** menu.
4. Select "***320***" for the ***Excitation***, and "***620***" for the ***Emission*** from the ***Wavelengths*** menu. Select "***570 nm***" for the ***Auto Cutoff***.
5. Select "***320***" for the ***Excitation***, and "***665***" for the ***Emission*** from the ***Wavelengths*** menu. Select "***630 nm***" for the ***Auto Cutoff***.
6. Set "***100***" for the ***Readings*** and "***Auto***" for the ***PMT***. 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.

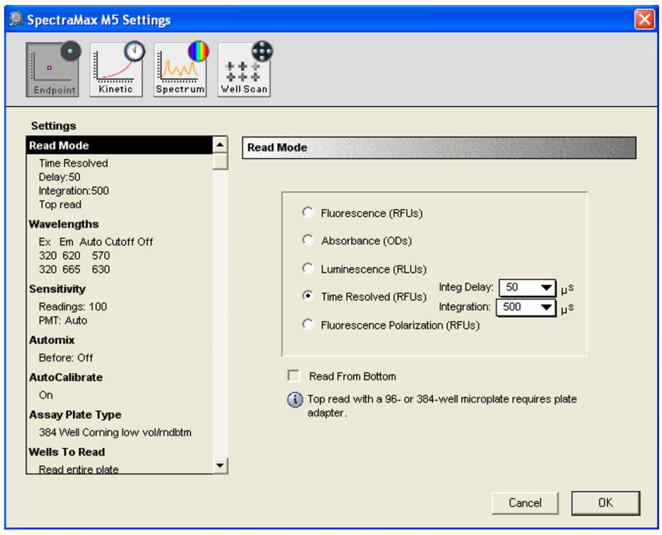

Figure 2. Screen Snapshot of Settings Dialog Box

Sample 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 Molecular Devices' FlexStation® 3 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 27nM 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 **High FRET Mixture** into wells P1-P12.
5. Dispense 10 µL of Low FRET Mixture into wells P13-P24.

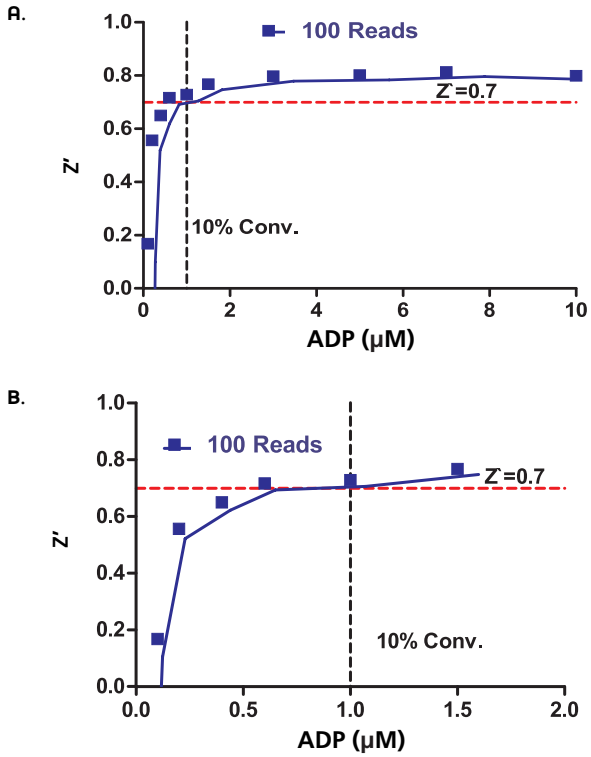

Figure 2. A). Z' values observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line.) Plate reader set to 100 reads.

Assay Performance, 10% Conversion 10 µM ATP			
Readings/Well	20	50	100
Z'-Factor at 10% ATP Conversion	0.55	0.69	0.73

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® 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® 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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