Application Note

BellBrook Labs

Optimized Settings to Validate the Molecular Devices Analyst® GT/HT Microplate Readers with the Transcreener® Fluorescence Intensity Assays.

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This Application Note describes the optimal instrument parameters used to validate the Analyst® GT/HT 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.

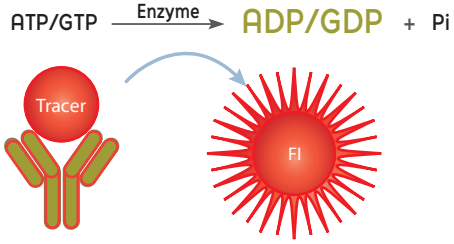

Figure 1. Transcreener® FI Assay Principle

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 number of 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 integration times 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.***

Analyst® GT/HT Information

- Standard 25mm filters for FP, TRF, TR-FRET, FI, and Absorbance (separate PMT for Luminescence).
- Readout maximization powered by 2nd generation Smart Optics® system.
- HTS compatible, high-speed bidirectional stacker option.
- Seamless integration into robotic environments.
- Plate formats supported: 96- to 1536-well microplates.

Molecular Devices

Instrument Settings

Instrument Wavelength Settings	
EXC Wavelength & Bandwidth	577/10 nm
EMS Wavelength & Bandwidth	625/30 nm
Dichroic Mirror	50:50 Beamsplitter
Optimized Analyst® GT Settings	
Lamp	Continuous
Z-Height	2 mm
Settle Time	10 ms (in advanced set up)
Attenuator Mode	Out
Raw Data Units	Counts/sec
Target SD/well	Not Set
Integration Time	Variable

Table 1. Recommended Analyst® GT Instrument Settings

Instrument Setup

The AnalystHost application provides a graphical user interface for setting up the reader and running assays. Proceed with the following steps to optimize the instrument:

1. Open the AnalystHost software, and select "***Detection***" from the ***Methods*** menu. Then select "***New***".
2. Select "***Fluorescence Intensity***" from the dropdown list and input a name for the method.
3. Click "***OK***" to view the ***Define and Edit Methods*** screen. The new method will be assigned the default parameters for the selected method type.
4. Do not modify ***Continuous Lamp, Raw Data Units of Counts/Sec*** or ***Attenuator Mode Out*** from the default settings. ***Continuous Lamp*** is recommended for highest sensitivity in the fluorescence intensity detection mode.
5. Select the appropriate microplate format being used from the ***Plate*** dropdown menu.
6. Select "***New***" from the ***Plates*** menu, and input the plate name and number of wells. Click "***OK***" and input the microplate dimensions (in millimeters) in the microplate schematic. Click "***OK***" to save.

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 Molecular Devices' Analyst® GT 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.

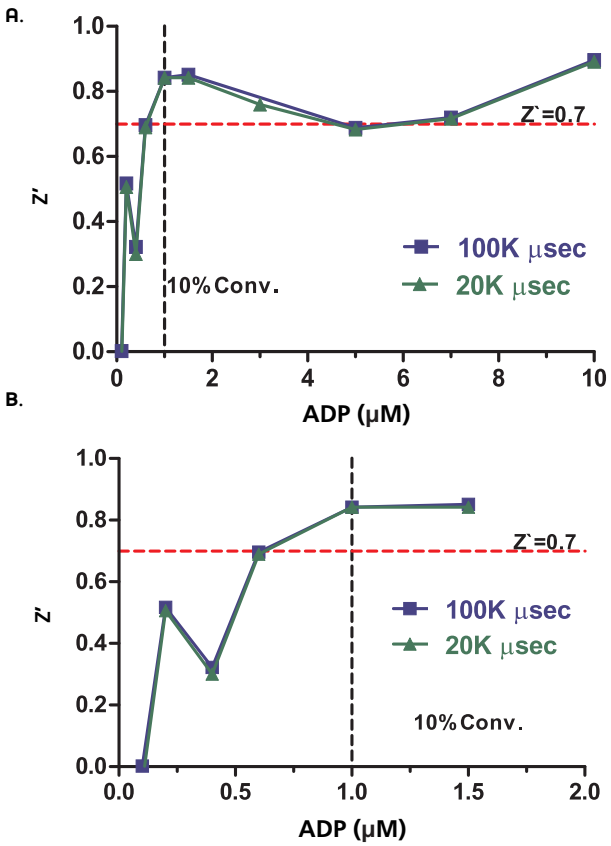

Figure 2. A). Z' values observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line.) Plate reader set at 20,000 or 100,000 µs integration time.

Assay Performance, 10% Conversion 10 µM ATP		
Integration Time	20K	100K
Read Time (minutes)	2:00	2:56
Z'-Factor at 10% ATP Conversion	0.84	0.84

Table 2. Assay Performance with Various Instrument Settings

Conclusions

This application note demonstrates the validation of the Molecular Devices Analyst® GT and HT 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

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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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