Application Note

BellBrook Labs

Optimized Settings to Validate the Molecular Devices Analyst® GT/HT Microplate Readers with the Transcreener® TR-FRET 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 TR-FRET (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 immunoassay 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.
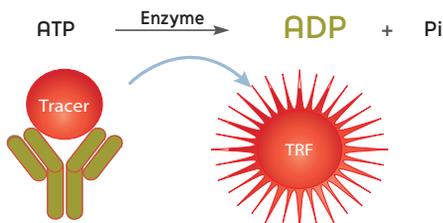

Figure 1. Transcreener® TR-FRET Assay Principle

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 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® TR-FRET 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	330/80 nm
EMS Wavelength & Bandwidth	665/7.5 nm
EMS Wavelength & Bandwidth	620/7.5 nm
Dichroic Mirror	380

Optimized Analyst® GT Settings	
Lamp	Flash
Z-Height	2 mm
Settle Time	25 ms (in advanced set up)
Time Between Readings	2 msec
Integration Time	500 µsec
Raw Data Units	Counts/sec
Target SD/well	Not Set
Readings/well	100
Delay After Flash	50 µsec

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 "*TR-FRET*" 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. Set the *Lamp* to *Flash Mode*.

4. Do not modify *Raw Data Units of Counts/Sec* or *Attenuator Mode Out* from the default settings.

5. Select the approprate microplate format being used from the *Plate* dropdown menu.

6. Set the *Z height* to "*2 mm*", *Time Between Readings* to "*2 msec*", *Integration Time* to "*500 µsec*", and *Readings/Well* to "*100*".

7. 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 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 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 C, 8 nM ADP^2 Antibody-Tb, and 27 nM of ADP HiLyte 647 Tracer.

High FRET Mixture - 8 nM ADP^2 Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 µM ATP in 1X Stop & Detect Buffer C

Low FRET Mixture - 8 nM ADP^2 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.

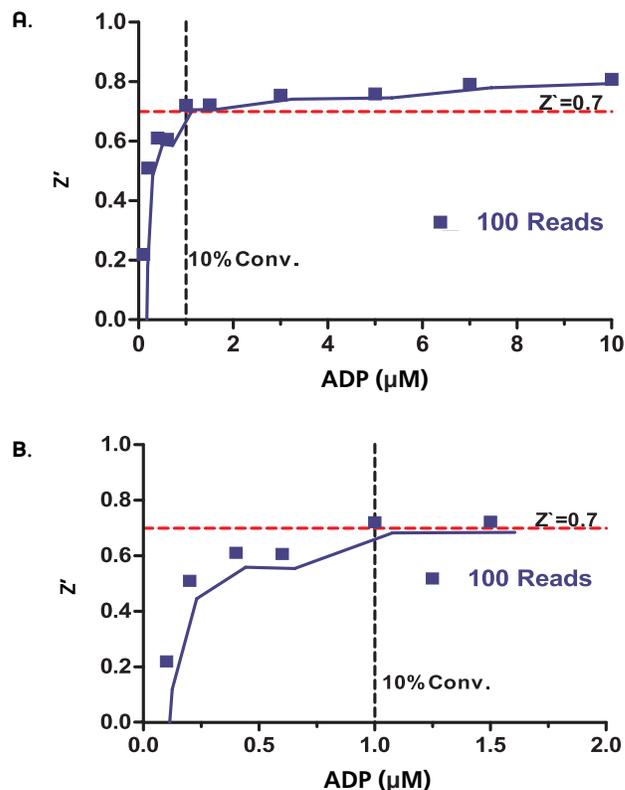5. Dispense 10 µL of Low FRET Mixture into wells P13-P24.

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 minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line.) Plate reader set at 100 Reads/Well.

Assay Performance, 10% Conversion 10 µM ATP		
Readings/Well	20	100
Read Time (minutes)	5:13	9:28
Z'-Factor at 10% ATP Conversion	0.55	0.72

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® 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.

<u>Phone Orders</u>:
608.443.2400
866.3137881

<u>Fax Orders</u>:
608.441.2967

<u>Email Orders</u>:
info@bellbrooklabs.com

Related Products

Transcreener® ADP^2 FP Assay	3010-1K
Transcreener® ADP^2 FI Assay	3013-1K
Transcreener® ADP^2 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.

Analyst® is a registered trademark of Molecular Devices.

LanthaScreen® Terbium is a registered trademark of Invitrogen (Life Technologies).

HiLyte Fluor™ is a trademark of Anaspec.

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.

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