Application Note

BellBrook Labs

Optimized Settings to Validate the BMG LABTECH PHERAstar Plus/FS HTS Microplate Readers with the Transcreener® Fluorescence Intensity Assays.

Meera Kumar[1], Brad Larson[1], Franka Ganske[2] and EJ Dell[2]
[1]BellBrook Labs, Madison, WI, USA; [2]BMG Labtech, Durham, NC, USA.

This Application Note describes the optimal instrument parameters used to validate the BMG LABTECH PHERAstar Plus/FS 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.

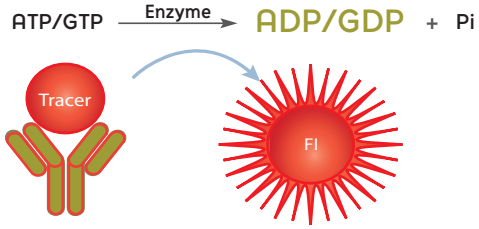


Figure 1. Transcreener® FI Assay Principle

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.***

PHERAstar Plus/FS Information

- Eight detection modes, separate measurement electronics, 5 PMTs, and assay specific optic modules.
- Equipped to read 6- to 1536- well plates.
- SDE decreases read times and increases accuracy as measured by %CV and Z' values.

BMG LABTECH

Instrument Settings

Instrument Wavelength Settings	
Optical Module	Transcreener FI Application Specific Module (EXC: 580/EMS: 620)

Optimized PHERAstar Settings	
Detector Gain 1	Should be Adjusted
Measuring Height	11.3 mm
Positioning Delay	0.1 sec
Flash Number	30-100

Table 1. Recommended PHERAstar Instrument Settings

Instrument Setup

To install the Transcreener-specific FI Optical Module, refer to http://www.bmglabtech.com/technology/optic-modules.cfm for a detailed protocol. Once the Transcreener-specific Optical Module has been installed, proceed with the following steps to optimize the detector gains and Z-height focus:

1. Select the "***Focus and Adjustment***" tab from the ***Measurement*** screen.
2. For optimization purposes, select a well containing ***only Free Tracer*** from the plate layout.
3. Select "***Focus Adjustment***" and "***Gain Adjustment***".
4. Select "***Start Adjustment***" to begin the optimization process.

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 ***Fluorescence Intensity*** screen is shown in Figure 2.

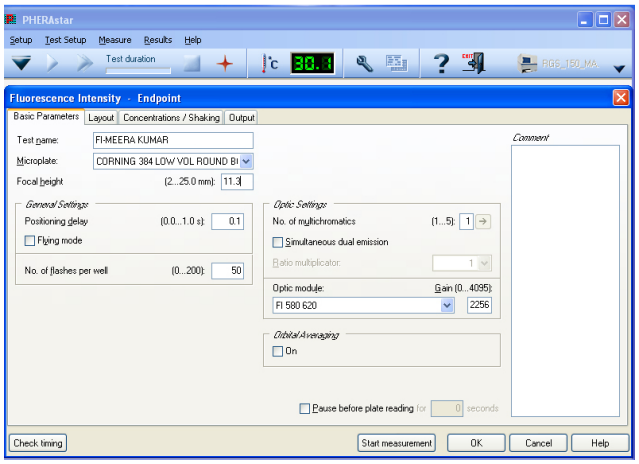


Figure 2. Screen Snapshot of Start Measurement Dialog Box

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 PHERAstar Plus HTS 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 ADP^2 Antibody-IRDye®QC-1.

Free Tracer - 1X Stop & Detect Buffer B and 8 nM ADP Alexa594 Tracer.

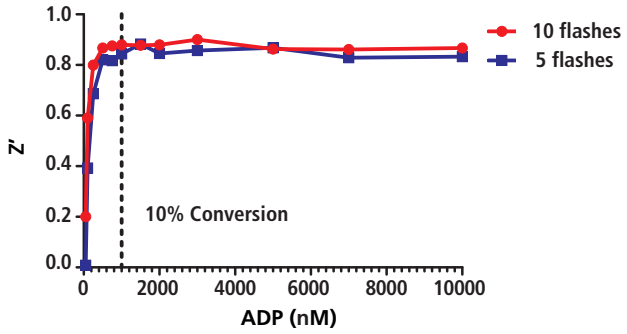
Buffer Blank - 1X Stop & Detect Buffer B and 10 µg/mL ADP^2 Antibody-IRDye®QC-1.

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.

A.



B.

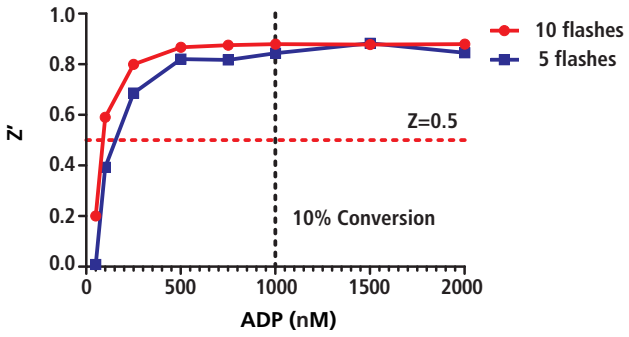


Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set at 10 flashes or 5 flashes.

Assay Performance at 10% Conversion 10 µM ATP					
Flashes	1	5	10	20	50
Read Time (minutes)	1:10	1:18	1:25	1:39	2:19
% CV at 10% ATP Conversion	7.01	3.34	2.73	1.92	2.23
Z'-Factor at 10% ATP Conversion	0.69	0.85	0.88	0.91	0.91

Table 2. Assay Performance with Various Instrument Settings

Conclusions

This application note demonstrates the validation of the BMG LABTECH PHERAstar Plus and PHERAstar FS instruments for use with the Transcreener® FI Assays. By utilizing the optimized instrument settings suggested within this Application Note, Z' values > 0.7 at 10% conversion 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

For technical information, please contact:

Meera Kumar, Applications Scientist
Tel: 608.443.2400
Toll-Free: 866.313.7881
Email: meera.kumar@bellbrooklabs.com

References & Notes

Transcreener® HTS Assay Platform is a patented technology of BellBrook Labs.

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

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