

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

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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 FP (3010) Transcreener® AMP/GMP (3006)
 Transcreener® UDP FP (3007) Transcreener® GDP FP (3009)*

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® FP Assays are a single step, competitive immunoassay for direct detection of nucleotides with a far red fluorescence polarization (FP) readout. The reagents for all of the assays are a far red Tracer bound to a highly-specific monoclonal/polyclonal antibody. Nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody, leading to increased rotational freedom and results in a decrease in polarization (Figure 1). The use of a far red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® FP Assays are designed specifically for HTS with a single addition, mix-and-read format.

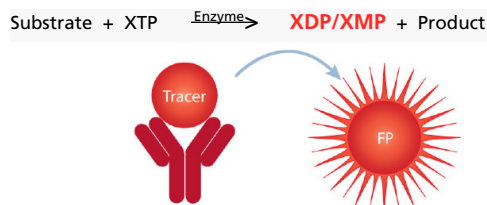


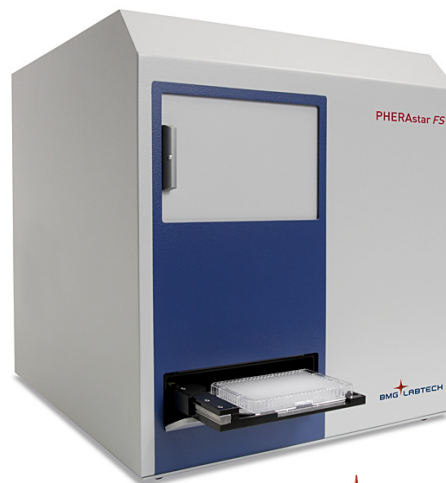
Figure 1. Transcreener® FP Assay Principle

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® FP Assays, a $Z' > 0.7$ and a $\Delta \text{mP} > 120$ at 10% conversion of 10 μM ATP was required.*

PHERAstar Plus/FS Information

- Multidetector HTS microplate reader with advanced fluorescence polarization capabilities.
- Full modularity in all detection modes covered by two matched pairs of PMT's and optimized assay specific optical modules.
- Simultaneous dual emission (SDE) detection for fastest read times and highest sensitivity.
- Assay flexibility given by precise temperature control and multimode shaking capabilities.




Instrument Settings

Instrument Wavelength Settings	
Optical Module	Transcreener FP Application Specific Module (EXC: 590/EMS: 675)
Optimized PHERAstar Plus/FS Settings	
Detector Gain 1 and 2	Should be Adjusted
Measuring Height	11.5 mm
Positioning Delay	0.1 sec
Flash Number	30-100

Table 1. Recommended PHERAstar Instrument Settings

Instrument Setup

To install the Transcreeper-specific FP Optical Module, refer to <http://www.bmglabtech.com/technology/optic-modules.cfm> for a detailed protocol. Once the Transcreeper-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". Within the "Gain Adjustment", set the target mP to *20 mP*.
4. Select "Use Advanced Options" and set the "Required Value" to *5%*. Since FP assays tend to have greater variability at higher gains, the selection of 5% ensures that the gain for the two emission channels of the Free Tracer does not yield counts >5% of the maximum 260,000.
5. 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 *Start Measurement* screen is shown in Figure 2.

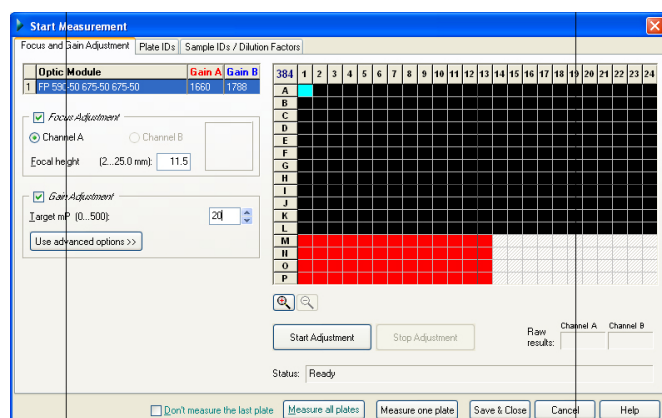


Figure 2. Screen Snapshot of Start Measurement Dialog Box

Sample FP Standard Curve

As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in mP values. Assay plates containing the 15-point standard curve were read on the PHERAstar Plus/FS HTS Microplate Reader.

Materials

ATP/ADP Mixture - 4 mM MgCl₂, 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, 4 nM ADP Alexa633 Tracer, and 14.8 μg/mL ADP² Antibody.

Free Tracer - 1X Stop & Detect Buffer B and 4 nM ADP Alexa633 Tracer.

Buffer Blank - 1X Stop & Detect Buffer B and 14.8 μg/mL ADP² Antibody.

For a detailed procedure on how to prepare a standard curve, please refer to the appropriate Transcreeper® Technical Manual (http://www.bellbrooklabs.com/transcreeper_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.

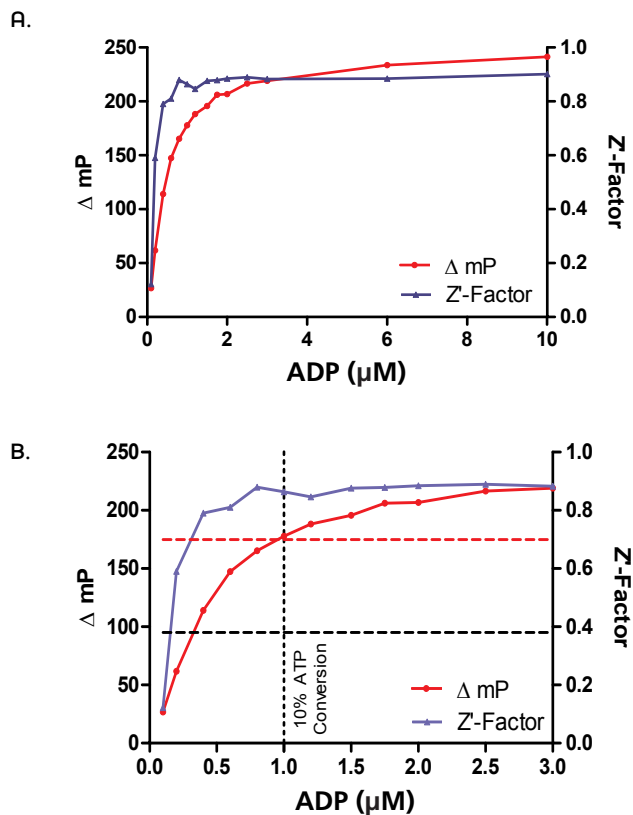


Figure 3. A). Z' values and Δ mP observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-2 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set at 100 flashes and 0.1 second positioning delay.

Assay Performance at 10% Conversion 10 μM ATP					
Flashes	30	50	75	100	125
Read Time (minutes)	1:55	2:22	2:56	3:29	4:03
Δ mP at 10% ATP Conversion	178	177	176	178	180
Standard Deviation at 10% ATP Conversion	8	6	7	4	4
Z'-Factor at 10% ATP Conversion	0.73	0.79	0.80	0.86	0.87

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® FP Assays. By utilizing the optimized instrument settings suggested within this Application Note, Z' values > 0.7 and ΔmP values > 120 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® 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.

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

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.

