

Optimized Settings to Validate the BMG LABTECH POLARstar Omega Microplate Reader with the Transcreener® Fluorescence Polarization Assays.

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This Application Note describes the optimal instrument parameters used to validate the BMG LABTECH POLARstar 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.

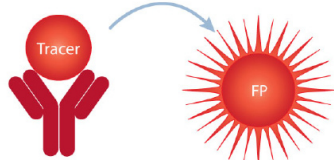
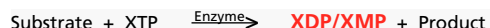


Figure 1. Transcreener® FP Assay Principle

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 $\Delta mP > 120$ at 10% conversion of 10 μM ATP were required*

POLARstar Omega Information

- Multidetector HTS microplate reader with Advanced Fluorescence Polarization capabilities.
- Tandem Technology - Unique BMG LABTECH technology uses ultra-sensitive filters and high speed UV/Vis spectrometers.
- 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	
Excitation Filter	610/10 nm
Emission Filter A	670/10 nm
Emission Filter B	670/10 nm
Optics	FP Optical Light Guide
Optimized POLARstar Omega Settings	
Detector Gain 1	2306
Detector Gain 2	2503
Positioning Delay	1 sec
Flash Number	150-200

Table 1. Recommended POLARstar Instrument Settings

Instrument Setup

The excitation filter, emission filters, and FP Optical Light Guide were installed prior to instrument evaluation. Once these components have been installed, proceed with the following steps:

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 "**Gain Adjustment**". Within the "**Gain Adjustment**", set the target mP to **20 mP**.
4. Select "**Use Advanced Options**" and set the "**Required Value**" to **2%**. Since FP assays tend to have greater variability at higher gains, the selection of 2% ensures that the gain for the two emission channels of the Free Tracer does not yield counts >2% 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 **Fluorescence Polarization** screen is shown in Figure 2.

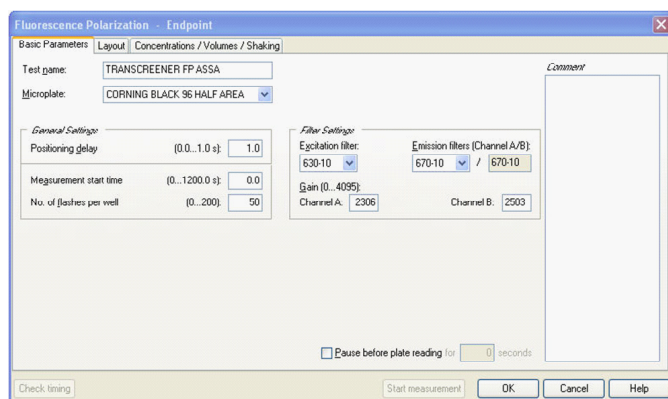


Figure 2. Screen Snapshot of Fluorescence Polarization Dialog Box

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 POLARstar Omega 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 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.

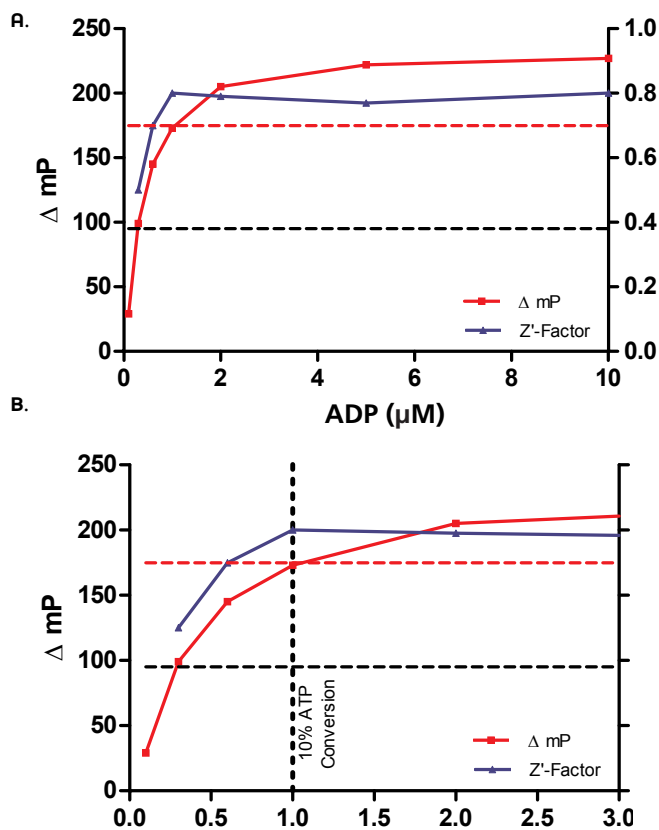


Figure 3. A) Z' values and Δ mP observed in a standard curve mimic conversion of 10 μM ATP to ADP. B) Zoomed view of the 0-3 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set at 150 flashes and 1.0 second positioning delay.

Assay Performance at 10% Conversion 10 μM ATP

Assay Performance at 10% Conversion 10 μM ATP			
Flashes	150	175	200
Read Time (minutes)	1:55	2:22	2:56
Δ mP at 10% ATP Conversion	173	172	173
Standard Deviation at 10% ATP Conversion	5	8	6
Z'-Factor at 10% ATP Conversion	0.80	0.70	0.76

Table 2. Assay Performance with Various Instrument Settings

Conclusions

This application note demonstrates the validation of the BMG LABTECH POLARstar Omega instrument 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.

