Application Note

BellBrook Labs

Optimized Settings to Validate the BMG LABTECH CLARIOstar 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 CLARIOstar 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.

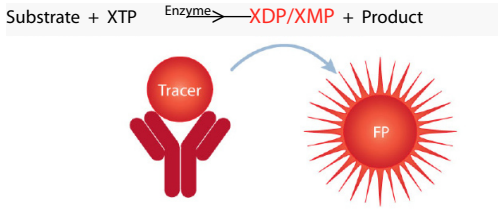


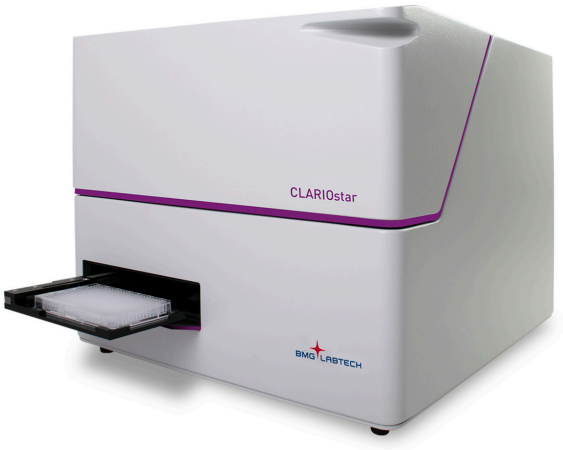
Figure 1. Transcreener® FP Assay Principle

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 100 µM ATP/ADP standard curve (24 replicates), as standard curves of this type mimic enzyme reactions. Starting with 100 µM ATP, ADP was added in increasing amounts and ATP is decreased proportionately, maintaining a total adenine nucleotide concentration of 100 µM. In order to validate an instrument for use with the Transcreener® FP Assays, a Z' > 0.7 and a Δ mP > 100 at 10% conversion of 10 µM ATP was required.

CLARIOstar Information

- High performance, modular, and upgradable instrument that performs all of the leading non-isotopic detection technologies
- Assay flexibility is given by Triple Detection Technology: Advanced LVF MonochromatorsTM, spectrometer, and filters
- LVF MonochromatorsTM have filter-like sensitivity and flexibility
- An integrated fluorophore library contains spectra for the most common fluorophores while offering recommended settings



BMG LABTECH

Instrument Settings

Instrument Wavelength Settings	
Optical Module	Transcreener FP Application Specific Module (EXC: 590/EMS: 675)

Optimized CLARIOstar Settings	
Filter Settings	590-50/ LP 640/ 675-50
Settling time	.3 sec
Number of flashes	50-200
Focus and Grain	Adjusted prior the measurement
Target mP	Was set to be 20mP for the free tracer (ADP Alexa633)

Table 1. Recommended CLARIOstar Instrument Settings

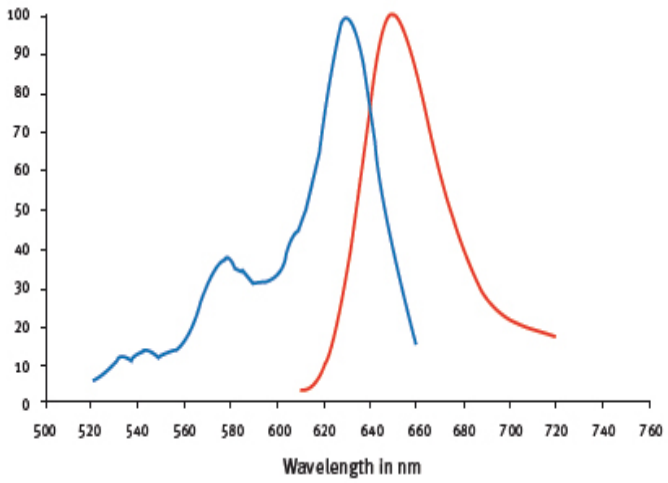


Figure 2. ADP Alexa633 tracer from BellBrook Labs Transcreener® ADP² assay was scanned on the CLARIOstar® using LVF Monochromator. The excitation of tracer was scanned between 520 and 660 nm, a constant emission wavelength of 690 nm was used. The emission was scanned between 610 and 720 nm at a fixed excitation wavelength of 580 nm.

Instrument Setup

Install the Transcreener FP filters in the CLARIOstar and make sure that the filters are set into the filter table. Proceed with the following steps to optimize the detector gains and Z-height focus:

1. Go to 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 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 3.

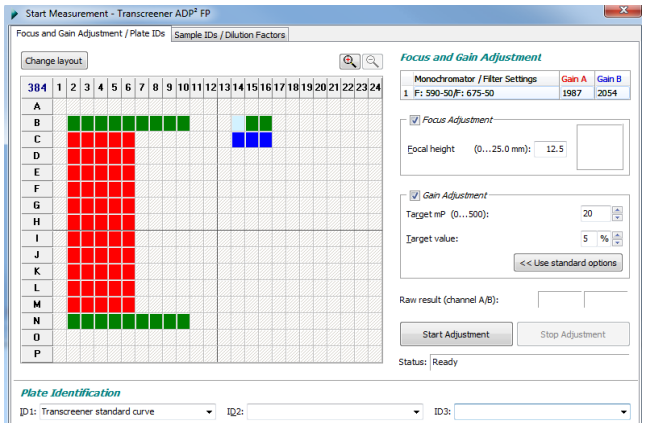


Figure 3. Screen Snapshot of Start Measurement Dialog Box

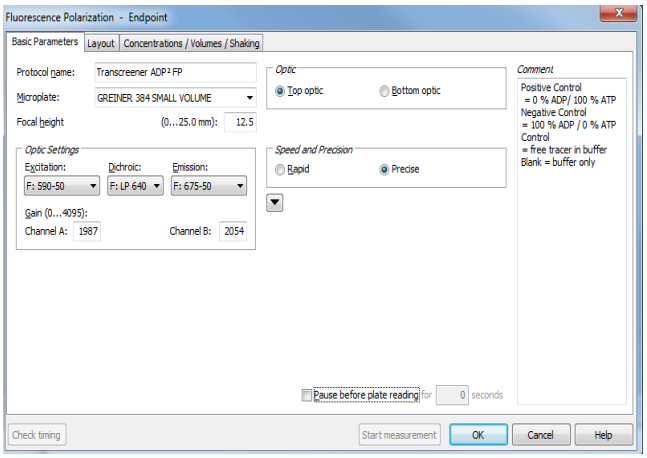


Figure 4. Screen Snapshot of Instrument Settings

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 CLARIOstar 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 100 µM).

ADP Detection Mixture - 1X Stop & Detect Buffer B, 4 nM ADP Alexa633 Tracer, and 1.08 µg/mL ADP² Antibody.

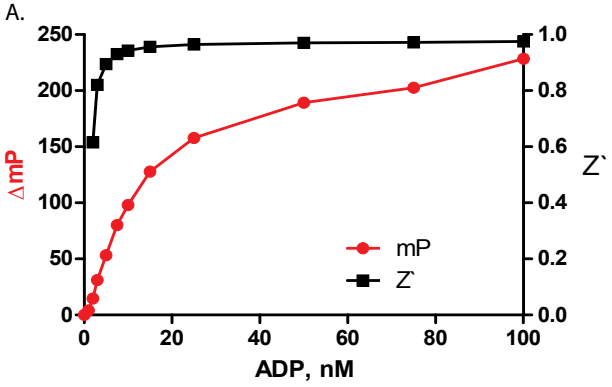
Free Tracer - 1X Stop & Detect Buffer B and 4 nM ADP Alexa633 Tracer.

Buffer Blank - 1X Stop & Detect Buffer B and 1.08 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.



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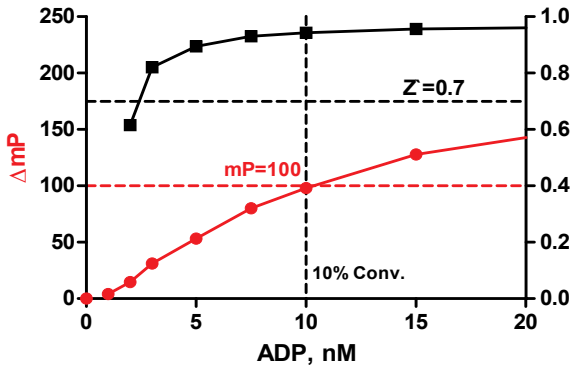

Figure 5. A). Z' values and Δ mP observed in a standard curve mimic conversion of 100 µM ATP to ADP. B). Zoomed view of the 0-20 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line).

Conclusions

This application note demonstrates the validation of the BMG LABTECH CLARIOstar 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 > 100 at 10% conversion are achievable.

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.

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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