

Optimized Settings to Validate the BMG LABTECH POLARstar/FLUOstar Omega Microplate Readers with the Transcreener® Fluorescence Intensity Assays.

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

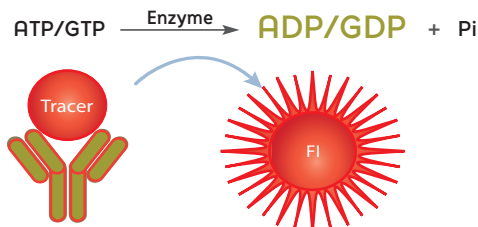


Figure 1. Transcreener® FI Assay Principle

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.

POLARstar/FLUOstar Omega Information

- Equipped with Tandem Technology that uses ultrasensitive filters and high speed UV/Vis spectrometers.
- Equipped to read 6- to 1536-well plates.
- Assay flexibility achieved by reagent injectors, precise temperature control, multi-mode shaking, and top/bottom reading.
- POLARstar Omega is capable of measuring FP, FI and TR-FRET, while FLUOstar Omega is capable of FI and TR-FRET readouts.



BMG LABTECH



Instrument Settings

Instrument Wavelength Settings	
Excitation Filter	580 nm/10 nm
Emission Filter	620 nm/10 nm
Optimized POLARstar/FLUOstar Omega Settings	
Detector Gain 1	should be adjusted
Measuring Height	11.3 mm
Flash Number	5-100

Table 1. Recommended POLARstar/FLUOstar Omega Instrument Settings

Instrument Setup

The Transcreeper FI filters were installed prior to instrument evaluation. Once these components have been installed, proceed with the following steps:

1. Select the "Fluorescence Intensity" tab from the *Measurement* screen.
2. Select "580" nm and "620" nm for the *Excitation* and *Emission* filters, respectively.
3. Select wells with *4 nM Tracer in 0.5 Stop & Detect Buffer B* 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.

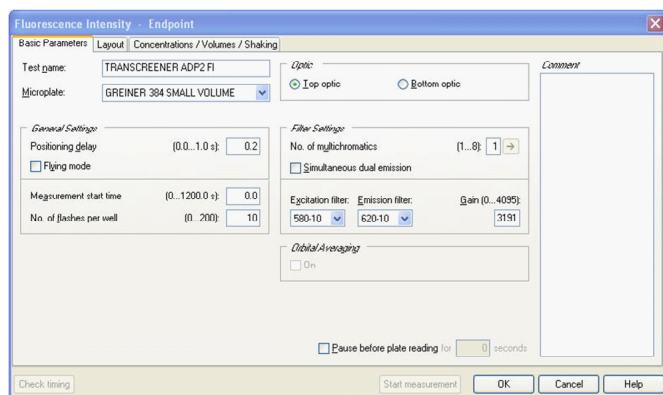


Figure 2. Screen Snapshot of Fluorescence Intensity Dialog Box

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 POLARstar/FLUOstar 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, 8 nM ADP Alexa594 Tracer, and 10 μg/mL ADP² Antibody-IRDye[®]QC-1.

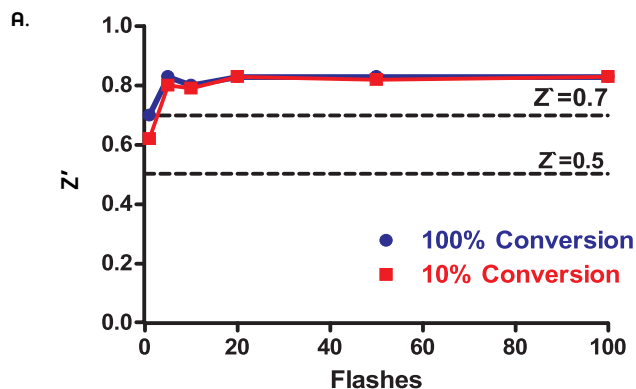
Free Tracer - 1X Stop & Detect Buffer B and 8 nM ADP Alexa594 Tracer.

Buffer Blank - 1X Stop & Detect Buffer B and 10 μg/mL ADP² Antibody-IRDye[®]QC-1.

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.



B.

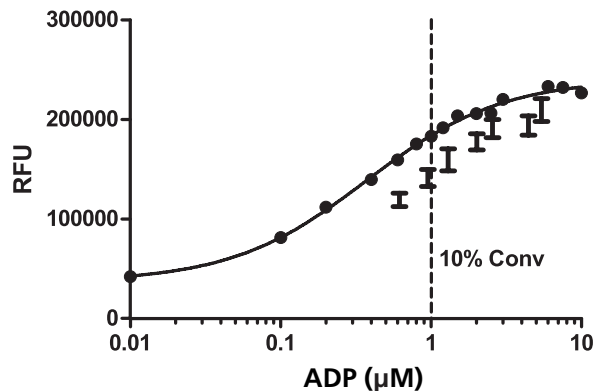


Figure 3. A) Z' values observed at 10% and 100% conversion of 10 μM ATP to ADP, as the number of flashes is varied (black dotted lines indicate 0.5 and 0.7 Z' values) B) 10 μM ATP/ADP standard curve shows the 10% ATP conversion validation point (black dotted line).

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	6.00	3.30	3.10	2.50	2.80
Z' -Factor at 10% ATP Conversion	0.62	0.80	0.79	0.83	0.82

Table 2. Assay Performance with Various Instrument Settings

Conclusions

This application note demonstrates the validation of the BMG LABTECH POLARstar/FLUOstar Omega instruments for use with the Transcreeper[®] 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® 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).

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

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.

