

Optimized Settings to Validate the BMG LABTECH PHERAstar Plus/FS HTS Microplate Readers with the Transcreener® TR-FRET Assays.

Meera Kumar¹, Brad Larson¹, Franka Ganske² and EJ Dell²
¹BellBrook Labs, Madison, WI, USA; ²BMG Labtech, Durham, NC, USA.

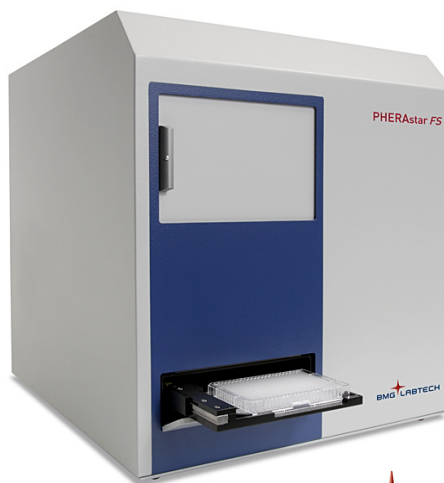
This Application Note describes the optimal instrument parameters used to validate the BMG LABTECH PHERAstar Plus/FS plate readers with the following assays:

Transcreener® ADP² TR-FRET (3011)

to determine the requirements for a $Z' > 0.5$. *In order to validate an instrument for use with the Transcreener® TR-FRET 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.




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® TR-FRET Assays are a single step, competitive immunoassay for direct detection of nucleotides with a far red time-resolved Förster-resonance-energy-transfer (TR-FRET) readout. The reagents for all of the assays are a far red Tracer bound to a highly-specific monoclonal antibody-Terbium conjugate. Excitation of the Terbium complex in the UV range (ca. 330 nm) results in energy transfer to the Tracer and emission at a higher wavelength (665 nm) after a time delay. Nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody, leading to a decrease in TR-FRET (Figure 1). The use of a red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® TR-FRET Assays are designed specifically for HTS with a single addition, mix-and-read format.

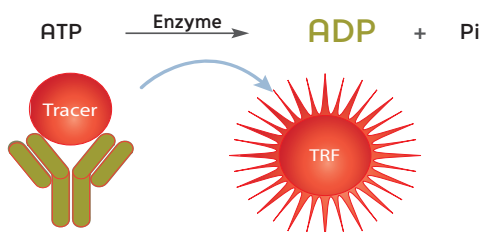


Figure 1. Transcreener® TR-FRET Assay Principle

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

Instrument Settings

Instrument Wavelength Settings

Optical Module	Transcreener Application Specific Module EXC: 337 nm EMS 1: 665 nm/EMS 2: 620 nm
----------------	--

Optimized PHERAstar Settings

Integration Start and Time	50 μs
Measuring Height	10.7 mm
Positioning Delay	0.1 sec
Flash Number	variable

Table 1. Recommended PHERAstar Instrument Settings

Instrument Setup

To install the Transcreeper Application Specific Module, refer to <http://www.bmglabtech.com/technology/optic-modules.cfm> for a detailed protocol. Once this module has been installed, proceed with the following steps to optimize the instrument:

1. Select the "Focus and Adjustment" tab from the *Measurement* screen.
2. Select "Simultaneous Dual Emission" and set the *Multiplicator* to 10,000.
3. For optimization purposes, select a well containing *Low Fret* from the plate layout.
3. Select "Focus 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 *Time Resolved Fluorescence* screen is shown in Figure 2.

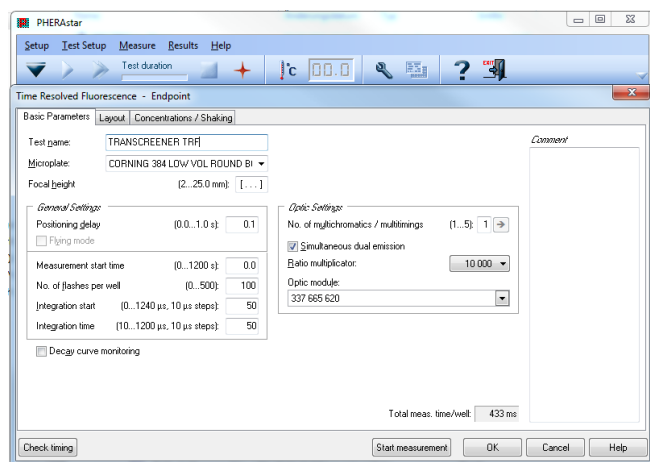


Figure 2. Screen Snapshot of Time Resolved Fluorescence Dialog Box

Sample TR-FRET Standard Curve

As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in FRET. Assay plates containing the 15-point standard curve were read on the PHERAstar Plus 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 C, 8 nM ADP² Antibody-Tb, and 27 nM ADP HiLyte647 Tracer.

High FRET Mixture - 8 nM ADP² Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 μM ATP in 1X Stop & Detect Buffer C.

Low FRET Mixture - 8 nM ADP² Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 μM ADP in 1X Stop & Detect Buffer C.

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 the High FRET mixture into wells P1-P12.
5. Dispense 10 μL of the Low FRET mixture into wells P13-P24.

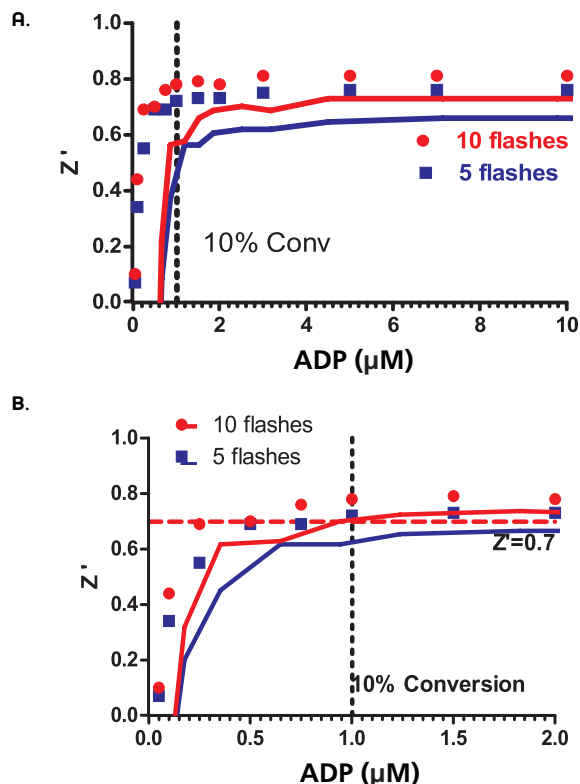


Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-2 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set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

Assay Performance at 10% Conversion 10 μM ATP							
Flashes	1	5	10	25	50	100	200
Read Time (minutes)	1:16	1:22	1:28	1:48	2:22	3:29	5:44
% CV at 10% ATP Conversion	14.1	8.3	4.91	4.52	2.5	2.38	1.56
Z'-Factor at 10% ATP Conversion	0.53	0.72	0.78	0.77	0.79	0.78	0.88

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 Transcreeper® TR-FRET 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

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.

Transcreener® is a registered trademark of BellBrook Labs.

LanthaScreen® Terbium is a registered trademark of Invitrogen (Life Technologies).

HiLyte Fluor™ is a trademark of Anaspec.

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.

