Application Note

BellBrook Labs

Optimized Settings to Validate the BioTek® Synergy™ H4/2/4 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 BioTek® Synergy™ H4/2/4 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.

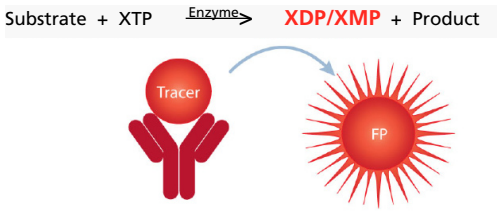

Figure 1. Transcreener® FP Assay Principle

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 Δ mP > 120 at 10% conversion of 10 µM ATP were required.*

Synergy™ Information

Synergy™ H4 Model

- Hybrid Technology: High-performance filter-based detection, flexible monochromator based detection.
- Compatible with Take3™ Multi-volume Plate with 2 µL microspots for low volume assays.
- Quadruple monochromator system includes variable bandpass selection for added flexibility.
- Filter-based optics use dichroic mirrors for fast read speeds and best performance.

Synergy™ 2 Model

- Detection modes available as individual modules.
- Plate formats: 6- to 1536-well plates.
- Unique combination of monochromator, filters and dichroic mirrors for best performance in all modes.
- 3 broad spectrum light sources for optimal sample illumination.
- Powered by Gen5™ Data Analysis Software.

BioTek®

Instrument Settings

Instrument Wavelength Settings	
EXC Wavelength and Bandwidth	620/40 nm
EMS Wavelength and Bandwidth	680/30 nm
Optics	660 nm (Top Position)

Optimized Synergy™ H4 Settings	
Sensitivity	Auto (106)
Position Delay	0 ms
Lamp	Xenon or Tungsten
Flash Number	>3

Table 1. Recommended BioTek® Synergy™ Instrument Settings

Instrument Setup

1. Open the Gen5™ software, and select "***Protocol***" from the ***Create a New Item*** menu. If a protocol already exists to run a Transcreener® FP Assay, select "***Experiment***" instead of "***Protocol***", and proceed to step 10.
2. Double click on "***Procedure***" and select "***Corning 384 Low Volume Round Bottom***" from the ***Plate Type*** dropdown list.
3. Click on "***Read***" to modify the plate reading parameters and select "***Fluorescence***" from the ***Read Type*** dropdown menu.
4. Check ***"Use Filter Wheels***" and "***Polarization***". Select the appropriate lamp from the ***Light Source*** dropdown menu.
5. Click on the small box to the right of the ***Read Speed*** dropdown menu and enter ***0 msec*** for the ***Delay After Plate Movement***. Input the prefered number of measurements to obtain in the ***Measurements Per Data Point*** box. Select "***High (More Sensitivity)***" for the ***Lamp Energy*** and click "***OK***" when finished.
6. Select ***"620/40"*** from the ***Excitation*** dropdown menu, and ***"680/30"*** from the ***Emission*** dropdown menu. Select "***Top 660 nm***" from the ***Optics Position*** dropdown menu. The T***op Probe Vertical Offset*** can remain at the default ***7.00 mm*** setting.
7. Select "***Options***" in the ***Sensitivity*** portion of each ***Read Step***. Check "***Automatic Sensitivity Adjustment***", and then select "***AutoScale***". Click "***OK***" when finished.
8. Double click on "***Plate Layout***", select "***Sample***" from the ***Type*** dropdown menu, and select the wells of the plate to be read. Click "***OK***" when finished.
9. To save the file, click "***Save***" when finished. The protocol is now ready for use and can be accessed from the ***File*** menu by selecting, "***New Experiment***".
10. Select the protocol that was just created. If a protocol was created previously, select that protocol. Click on "***Read Plate***" and then on "***Read***".

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 ***Read Step*** screen is shown in Figure 2.

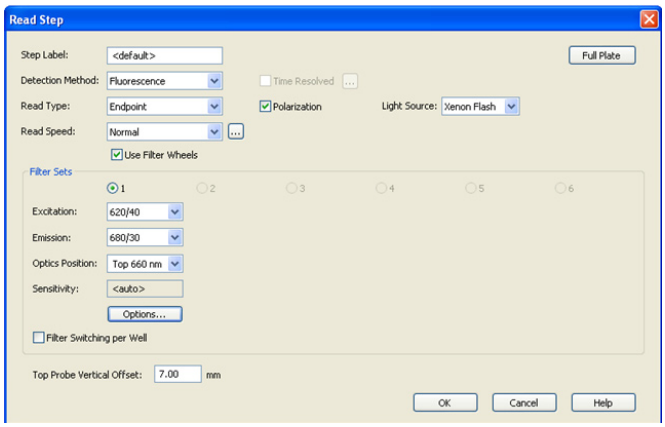

Figure 2. Screen Snapshot of Gen5™ *Read Step* Dialog Box

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 Synergy™ H4 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 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.

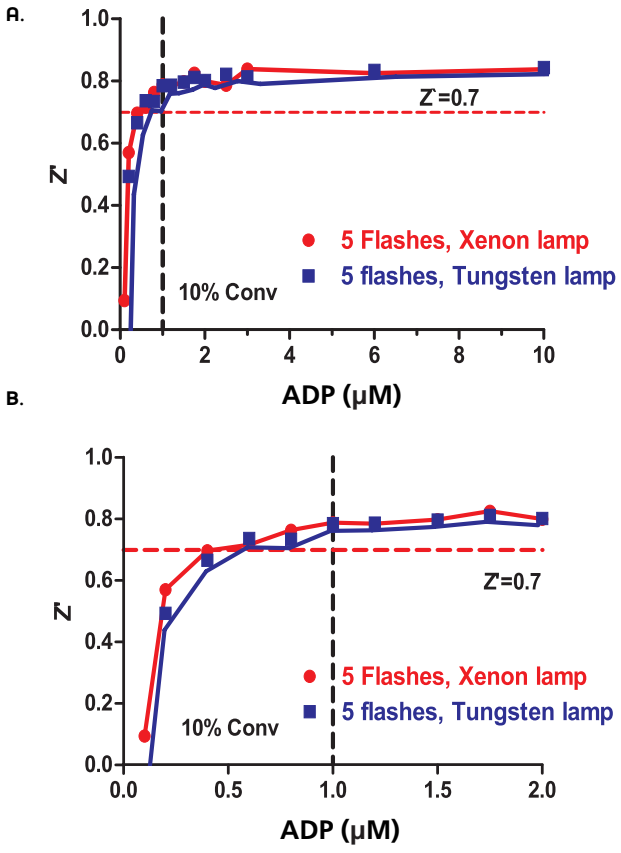

Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set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

Assay Performance, 10% Conversion 10 µM ATP: Xenon Flash Lamp							
Flashes	1	3	5	7	9	11	12
Read Time (minutes)	1:47	2:18	2:50	3:26	3:59	4:31	4:48
Δ mP at 10% ATP Conversion	168	162	162	163	162	164	163
Z'-Factor at 10% ATP Conversion	0.45	0.68	0.79	0.83	0.82	0.84	0.85

Assay Performance, 10% Conversion 10 µM ATP: Tungsten Flash Lamp							
Flashes	1	3	5	7	9	11	13
Read Time (minutes)	1:16	1:53	2:23	2:54	3:24	3:54	4:24
Δ mP at 10% ATP Conversion	158	160	163	160	162	162	162
Z'-Factor at 10% ATP Conversion	0.57	0.75	0.78	0.83	0.82	0.83	0.84

Table 2. Assay Performance with Various Instrument Settings

Conclusions

This application note demonstrates the validation of the BioTek® Synergy™ H4, Synergy™ 2 and Synergy™ 4 microplate readers for use with the Transcreener® FP Assays. By utilizing the optimized instrument settings suggested within this Application Note, Z' values > 0.7 and ΔmP > 120 are achievable.

References & Notes

Transcreener® HTS Assay Platform is a patented technology of BellBrook Labs.

Transcreener® is a registered trademark of BellBrook Labs.

Synergy™ is a trademark of BioTek.

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

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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