Application Note

BellBrook Labs

Optimized Settings to Validate the BioTek® Synergy™ H4/2/4 HTS Microplate Readers with the Transcreener® TR-FRET Assays.

Meera Kumar[1], Karen Kleman-Leyer[1], Brad Larson[2] and Xavier Amouretti[2]
[1]BellBrook Labs, Madison, WI, USA; [2]BioTek Instruments, Winooski, VT, USA.

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 TR-FRET Red Assay (3011)

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.

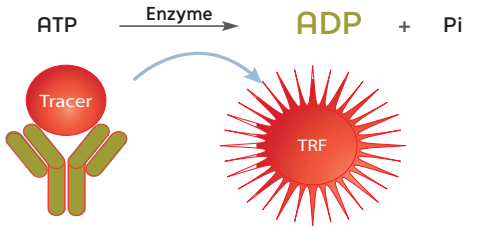

Figure 1. Transcreener® TR-FRET Assay Principle

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 to determine the requirements for a Z' > 0.5. *In order to validate an instrument for use with the Transcreener® TR-FRET Assay, a Z' > 0.7 at 10% conversion of 10 µM ATP was 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	340/30 nm
EMS Wavelength and Bandwidth	620/10 nm
EMS Wavelength and Bandwidth	665/8 nm
Optics	400 nm (Top Position)

Optimized Synergy™ H4 Settings	
Delay Before/After Plate Movement	100 µsec/0 msec
Data Collection Time	200 µsec
Sensitivity	Automatic
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® TR-FRET Assay, select "***Experiment***" instead of "***Protocol***", and proceed to step 11.
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 "***Time Resolved***". Next, click on the small box to the right of the ***Time Resolved*** check box. Enter ***100 µsec*** for the ***Delay Before Collecting Data***, and ***200 µsec*** for the ***Data Collection Time***. Click "***OK***" when finished.
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. For filter set #1, select ***"340/30"*** from the ***Excitation*** dropdown menu, and ***"620/10"*** from the ***Emission*** dropdown menu. For filter set #2, select ***"340/30"*** from the ***Excitation*** dropdown menu, and ***"665/8"*** from the ***Emission*** dropdown menu. Select "***Top 400 nm***" from the ***Optics Position*** dropdown menu for both filter sets. 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. If the whole plate will not be read, select "***Full Plate***", uncheck "***Use All the Wells (Full Plate)***" and then select the wells of the plate that will be read. Click "***OK***" when finished. Next, click "***OK***" to close the Read step, and "***OK***" to close the Procedure.
9. 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.
10. 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***".
11. 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.

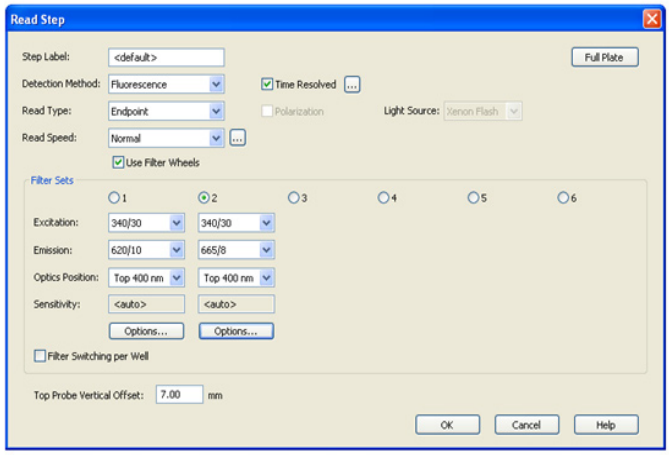

Figure 2. Screen Snapshot of Gen5™ *Read Step* Dialog Box

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

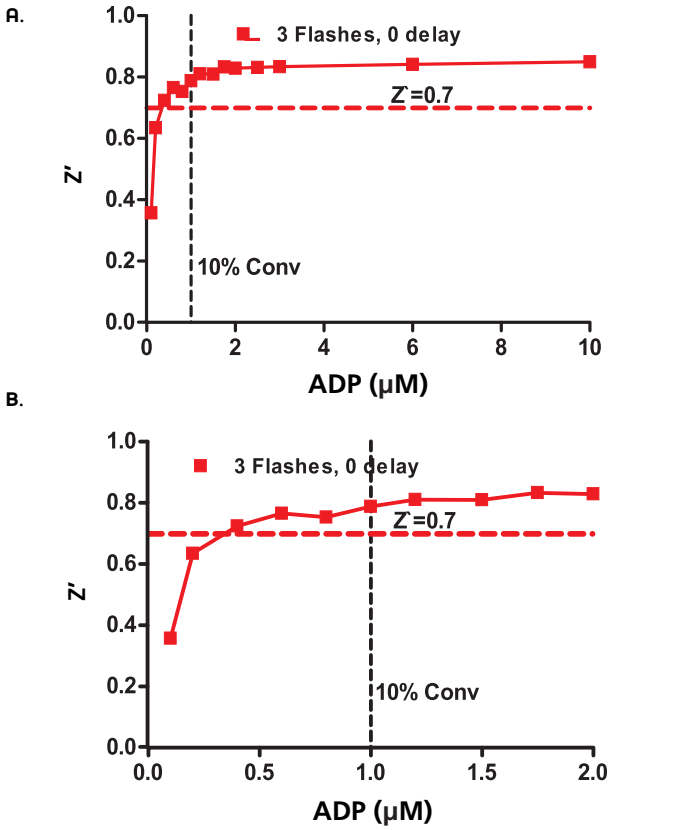

Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2 µM ADP section of the standard curve shows the Z' validation mininimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set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

Assay Performance at 10% Conversion 10 µM ATP								
Flashes	1	3	5	7	9	11	13	15
Read Time (minutes)	1:50	2:20	2:55	3:29	3:54	4:24	4:54	5:40
Z'-Factor at 10% ATP Conversion	0.66	0.78	0.82	0.82	0.85	0.86	0.86	0.84
% Standard Deviation at 10% ATP Conversion	1.8	1.5	0.9	1.0	0.9	0.8	0.7	0.9

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® TR-FRET Assays. By utilizing the optimized instrument settings suggested within this Application Note, Z' values > 0.7 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® 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

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.

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