

## Optimized Settings to Validate the BioTek® Synergy™ H4/2/4 HTS Microplate Readers with the Transcreener® TR-FRET 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® ADP<sup>2</sup> 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.

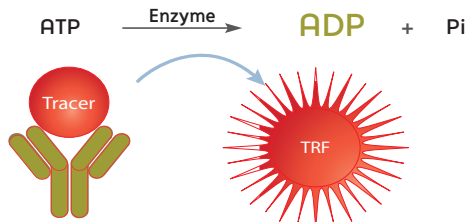


Figure 1. Transcreener® TR-FRET Assay Principle

### 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.



### 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 preferred 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 *Top 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.

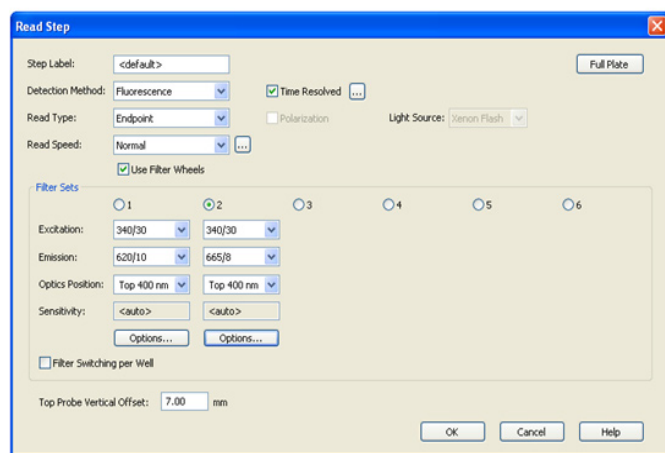


Figure 2. Screen Snapshot of Gen5™ Read Step Dialog Box

## 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<sub>2</sub>, 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 ADP<sup>2</sup> Antibody-Tb, and 27 nM ADP HiLyte647 Tracer

**High FRET Mixture** - 8 nM ADP<sup>2</sup> Antibody-Tb, 27 nM ADP HiLyte647 Tracer, 10 μM ATP in 1X Stop & Detect Buffer C

**Low FRET Mixture** - 8 nM ADP<sup>2</sup> 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](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.

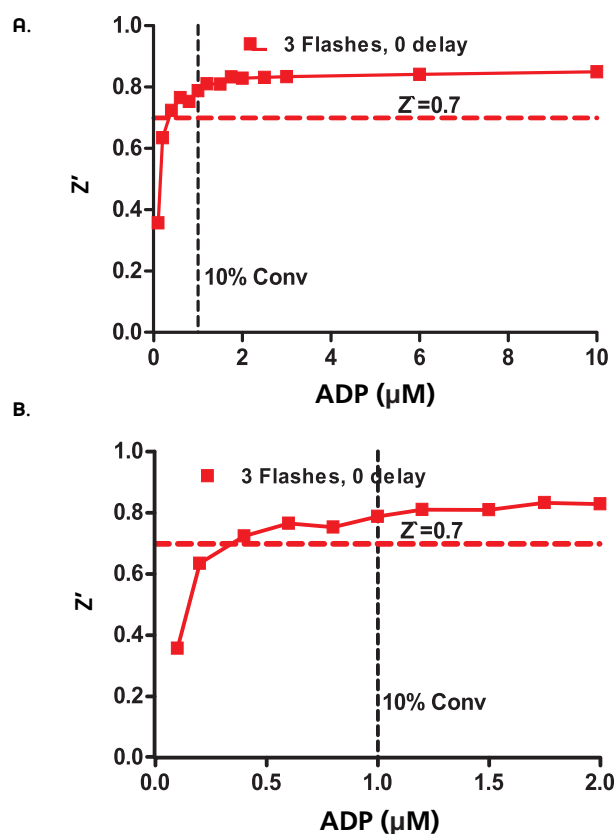


Figure 3. A). Z' values observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-2 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (black dotted line). Plate reader set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

#### Assay Performance at 10% Conversion 10 $\mu$ M 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](http://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](mailto:info@bellbrooklabs.com)

### Related Products

Transcreener® ADP <sup>2</sup> FP Assay.....	3010-1K
Transcreener® ADP <sup>2</sup> FI Assay.....	3013-1K
Transcreener® ADP <sup>2</sup> 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.

Synergy™ is a trademark of BioTek.

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.

