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Application Note

Utility of Synergy™ H4 Hybrid Multi-Mode Microplate Reader using both Monochromator- and Filter-Based Detection

Capabilities demonstrated with Fluorescence Polarization, Time-Resolved Fluorescence Resonance Energy Transfer, and Fluorescence Intensity Assays for the Measurement of ADP Accumulation

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Introduction

Multi-mode microplate readers are one of the more indispensable laboratory instruments used in life science research today. Advances in reader technology now allow for an incredible array of assays to be performed with readout based on the detection of a wide range of fluorophores, luminophores, and chromophores with spectral properties in the UV to IR. Most readers are either monochromator- or spectral filter-based. Monochromators offer the flexibility to read a virtually unlimited range of excitation and emission wavelengths, without the requirement for purchasing and swapping out a wide range of filters during the assay development phase. Monochromators also provide the ability to determine the spectra of chromophores and fluorophores for multiple applications including UV-Vis spectra of protein folding and assessing purity of isolated biomolecules. Filters provide significantly increased light throughput of desired analytical signals leading to improved sensitivity and facilitate detection modes such as fluorescence polarization (FP) and time-resolved fluorescence resonance energy transfer (TR-FRET). While each Reading technology is a new class of detectors that has advantages, the capabilities of the other detection system are sacrificed when purchasing one or the other. The Synergy H4 Hybrid Multi-Mode Microplate Reader eliminates the need for sacrificing flexibility or sensitivity by combining both detection modes into a single instrument.

Synergy™ H4 Hybrid Multi-Mode Microplate Reader

The BioTek Synergy™ H4 Hybrid Multi-Mode Microplate Reader was used to measure the fluorescence polarization, fluorescence intensity, and TR-FRET signals from the three different assay chemistries. The filter-based system, which incorporates deep blocking filters and a dichroic mirror was used with each assay, while the monochromator-based system was used to read the signal from the fluorescence intensity assay, as well as perform spectral scanning of one of the Transcreener® assay fluorophores.

Figure 1. Synergy™ H4 Hybrid Multi-Mode Microplate Reader. The instrument was used to quantify the fluorescent signal from all assay plates, as well as perform spectral scanning.

Transcreener® ADP^2 Assay Chemistries

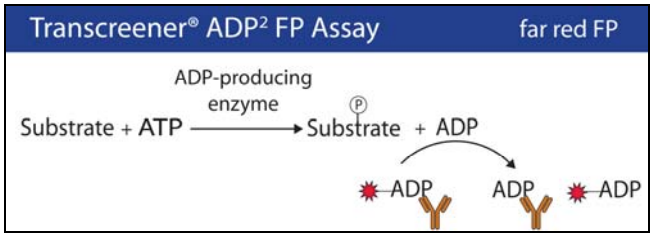

Figure 2. The Transcreener® ADP Detection Mixture comprises an ADP Alexa633 Tracer bound to an ADP^2 Antibody. The tracer is displaced by ADP, the invariant product generated during the enzyme reaction. The displaced tracer freely rotates leading to a decrease in fluorescence polarization.[1]

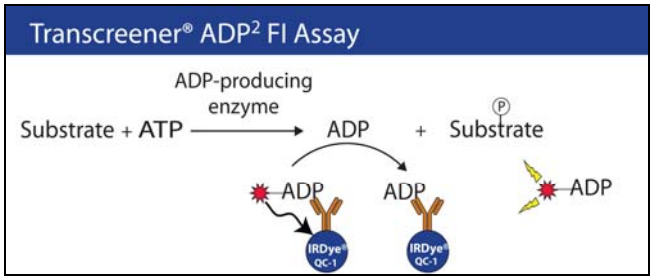

Figure 3. The Transcreener® ADP Detection Mixture comprises a quenched ADP Alexa594 Tracer bound to the ADP2 monoclonal antibody conjugated to an IRDye QC-1 quencher. The tracer is displaced by ADP, the invariant product generated during an enzyme reaction. The displaced tracer becomes un-quenched in solution leading to a positive increase in fluorescence intensity.[2]

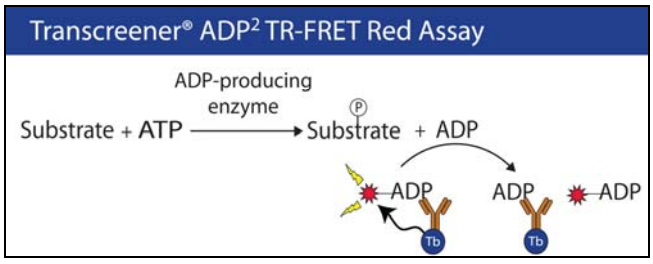

Figure 4. The Transcreener® ADP Detection Mixture comprises an ADP HiLyte647 Tracer bound to an ADP2 Antibody-Tb conjugate. Excitation of the terbium complex in the UV range (ca. 330 nm) results in energy transfer to the tracer and emission at a higher wavelength (665nm) after a time delay. ADP produced by the target enzyme displaces the tracer which causes a decrease in TR-FRET, and emission at 620nm.[3]

Materials and Methods

Here we show the utility of the Synergy™ H4 Hybrid Multi-Mode reader that combines the flexibility of monochromators with the sensitivity and speed of filter-based detection. Two double-grating monochromators are used to provide the highest stray light rejection, and continuous wavelength selection. Deep blocking filters and dichroic mirrors deliver high photon flux to the microplate wells and provide high signal-to-noise ratios in assays. We used three fluorescence-based assay technologies to test the capabilities of each detection system.

The assays utilize Fluorescence Polarization, Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET), or Fluorescence Intensity for the measurement of ADP accumulation. A 10 µM ADP/ATP standard curve was set up using increasing and decreasing concentrations of ADP and ATP, respectively, keeping the total adenine concentration constant in each well. The curve mimics the conversion of ATP to ADP in an enzyme reaction. Z'-Factor values and Relative Standard Deviation were determined using the 10 µM ATP/0 µM ADP and 9 µM ATP/1 µM ADP points on the curve. Scans of the ADP Alexa594 Tracer used in the Fluorescence Intensity assay were also performed. The data generated, as well as the results of the spectral scan, demonstrate the ability of the reader to yield high quality information using either detection system and its utility in today's life science research laboratory.

Component	Vendor	Part Number
384-Well Black, Low Volume, NBS Coated Assay Plate	Corning	3676
384-Well White, Low Volume, NBS Coated Assay Plate	Corning	3673
Transcreener® ADP^2 FP Assay	BellBrook Labs	3010-1K
Transcreener® ADP^2 FI Assay	BellBrook Labs	3013-1K
Transcreener® ADP^2 TR-FRET Assay	BellBrook Labs	3011-1K

Table 1. Project component list

Instrument Detection Component		BioTek Catalog #	Optimized Instrument Settings	
Transcreener® ADP^2 FP Assay				
Excitation Filter	620/40 nm	7082213	Lamp Energy	High
Emission Filter 1	680/30 nm	7082229	Measurements per Data Point	Variable
Dichroic	660 nm Cutoff	7137660	Top Probe Vertical Offset	7.00 mm
Transcreener® ADP^2 FI Assay				
Excitation Filter	590/20 nm	7082225	Lamp Energy	High
Emission Filter 1	620/10 nm	7082265	Measurements per Data Point	Variable
Dichroic	595 nm Cutoff	7138595	Top Probe Vertical Offset	7.00 mm
Transcreener® ADP^2 TR-FRET Assay				
Excitation Filter	340/30 nm	7082230	Delay/Data Collection Time	100 µs/ 200 µs
Emission Filter 1	620/10 nm	7082265	Measurements per Data Point	Variable
Emission Filter 2	665/8 nm	7082266	Lamp Energy	High
Dichroic	400 nm Cutoff	7138400	Top Probe Vertical Offset	7.00 mm

Table 2. Synergy™ H4 Transcreener® ADP^2 Assay Instrument Settings

Experimental Design

1. 15-Point Standard curves were setup containing various combinations of ATP and ADP, ranging from 10 µM ATP/0 µM ADP to 0 µM ATP/10 µM ADP. A constant concentration of 10 µM Adenine was maintained at each point included on the curve.
   a. The concentrations of ATP and ADP at each point on the curve mimic the conversion of ATP to ADP in an enzyme reaction.
   b. Two points on the curve were used for assay quality measurement. 10 µM ATP/0 µM ADP (100% ATP/0% ADP or 0% ATP Conversion) and 9 µM ATP/1 µM ADP (90% ATP/10% ADP or 10% ATP Conversion).
2. Twenty-Four 10 µL replicates of each point on the curve were added to a Corning 384-Well Low-Volume assay plate.
3. The appropriate assay detection mixture was then added to each plate at a 10 µL volume.
4. The plates were mixed for 30 seconds on an orbital shaker, covered, and incubated for 1 hour at RT, and then read on the Synergy™ H4.
5. Two data quality measurements were created with each test.
   a. Precision - %CV (Assessed using 10% ATP conversion std. curve point only)
   b. Assay Robustness – Z'-Factor (Assessed using both std. curve points)

Results and Discussion

Our intent in this application note is to demonstrate the capability of the Synergy™ H4 to read the signal from various fluorescent assay chemistries with acceptable speed, sensitivity, and accuracy.

Transcreener® ADP^2 FP Assay

Test 1. The Xenon lamp was used, along with variable flashes, to assess the affect of increasing read time on data quality.

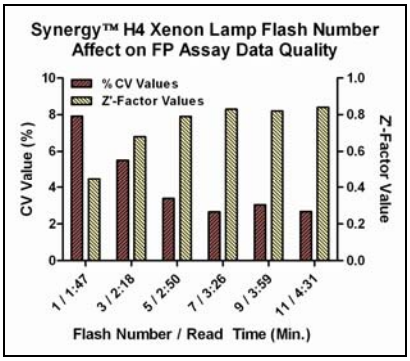

Figure 5. FP Assay Data Quality vs. Flash Number (Xenon Lamp)

Summary: Excellent Z' values can be achieved using as little as 3 flashes. Therefore high data quality can be seen while maintaining high-throughput.

Test 2. The Tungsten lamp was then tested, along with variable flashes, to compare data quality between the two lamps.

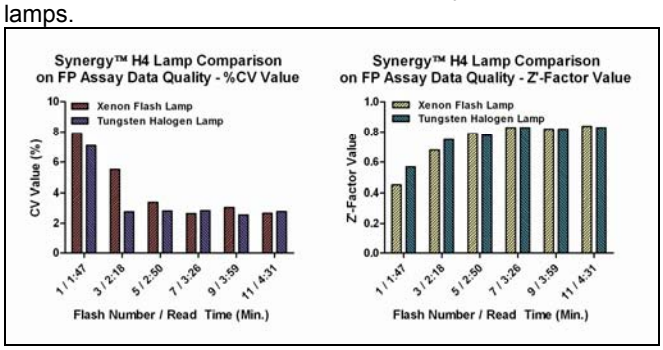

Figure 6. FP Assay Data Quality vs. Flash Number (Xenon and Tungsten Lamp Comparison)

Summary: The Tungsten lamp provides slightly better data quality at lower flash numbers, whereas the lamps perform equally well at 5 flashes or above.

Transcreener® ADP^2 TR-FRET Assay

Test 1. The Xenon lamp was used, along with variable flashes, to assess the affect of increasing read time on data quality.

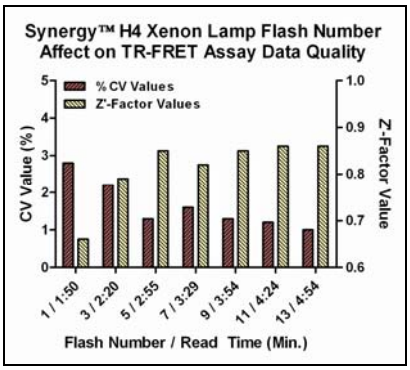

Figure 7. TR-FRET Assay Data Quality vs. Flash Number (Xenon Lamp)

Summary: Excellent Z' values can be achieved using as little as 3 flashes. Therefore high data quality can once again be seen while maintaining high-throughput.

Transcreener® ADP^2 FI Assay

Test 1. The Xenon and Tungsten lamps were tested, along with variable flashes, to compare data quality.

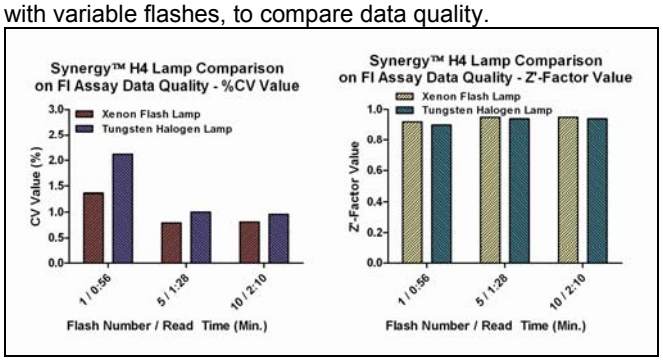

Figure 8. FI Assay Data Quality vs. Flash Number (Xenon and Tungsten Lamp Comparison)

Summary: Excellent Z' values can be achieved using as little as 1 flash. Therefore high data quality can be seen at the fastest read speeds.

Test 2. The monochromator system was tested using the Xenon lamp, along with variable flashes and bandpass settings, to compare data quality.

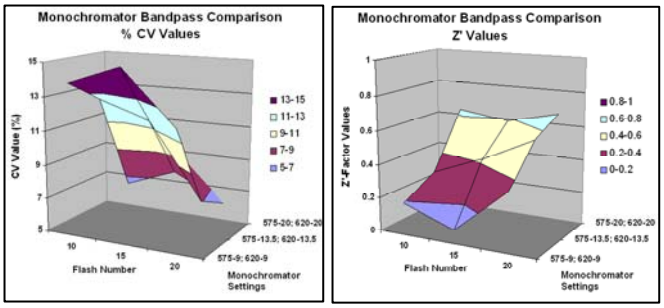

Figure 9. FI Assay Data Quality vs. Monochromator Bandpass Setting and Flash Number

Summary: At each read speed (flash number), data quality can be improved by using wider monochromator bandpass settings.

Test 3. Data quality with the filter and monochromator systems was compared using equivalent read times.

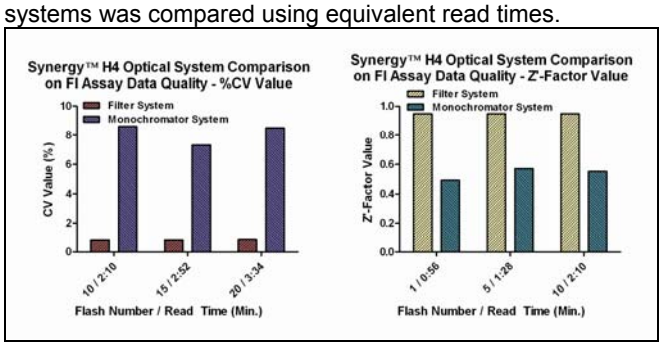

Figure 10. FI Assay Data Quality vs. Flash Number (Filter and Monochromator System Comparison)

Summary: At each read speed (flash number), data quality from the filter system exceeds that of the monochromator system. Therefore when performing applications where the highest quality data is necessary, use of the filter system is recommended.

Spectral Scanning Capability

An excitation and emission spectral scan was performed using the Alexa594 Tracer from the Transcreener® ADP^2 FI Assay. Excitation scans were performed from 450-620nm in 1nm increments, with emission set at 665nm. Emission scans were performed from 580-700nm in 1nm increments, with excitation set at 535nm. Four separate excitation and emission monochromator bandpass settings of 9, 13.5, 17.5, and 20 nm.

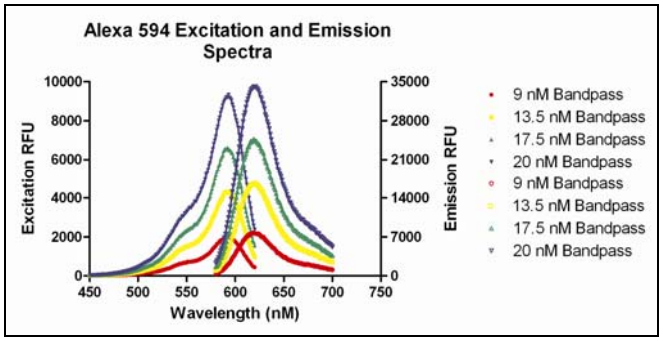

Figure 11. Spectral Scanning Results. RFUs (Y-axis) are plotted for each wavelength scanned (X-axis) using each of the four monochromator bandpass settings tested.

Summary. By examining the graph of the data, it is evident that excellent spectral scanning results can be achieved with the instrument. Wider bandpass settings can be used for fluors with lower energy, while narrower bandpass settings can be used with fluors possessing narrow Stokes shift.

Conclusions

1. The filter system of the Synergy™ H4 provides excellent data quality with the most common fluorescent assay outputs, using read speeds that maintain high-throughput.

2. The monochromator system provides excellent spectral scanning capabilities, as well as the ability to test a variety of wavelength settings with different fluorophores.

3. The ease of setup, and robustness of the Transcreener® ADP^2 assays allow them to be simply and accurately read on the Synergy™ H4.

4. The flexibility and sensitivity of the Synergy™ H4 make the instrument an excellent choice for use in today's life science research laboratory.

References

[1] BellBrook Labs Transcreener® ADP^2 FP Assay Technical Manual.

[2] BellBrook Labs Transcreener® ADP^2 FI Assay Technical Manual.

[3] BellBrook Labs Transcreener® ADP^2 TR-FRET Red Assay Technical Manual.

Rev. 2/24/10, AN022410_06