Application Note

Optimizing Performance of Transcreener® Fluorescent Polarization Assays with the SpectraMax Paradigm Microplate Reader

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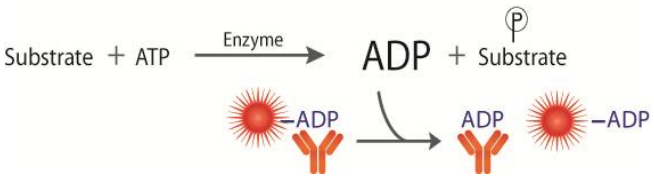
Introduction

This application protocol describes the optimal instrument parameters used to validate the SpectraMax® Paradigm® Microplate Reader with the following assays from BellBrook Labs:

- Transcreener ADP^2 FP (3010)
- Transcreener AMP/GMP (3006)
- Transcreener UDP FP (3007)
- Transcreener GDP FP (3009)

Transcreener® is a universal, high throughput biochemical assay platform based on the detection of nucleotides, which are formed by thousands of cellular enzymes. Many of these enzymes catalyze the covalent regulatory reactions that are central to cell signaling; e.g., phosphorylation, methylation, and are of high interest as therapeutic targets.

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 that binds 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 decreased 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.



The SpectraMax Paradigm Multi-Mode Microplate Reader offers dual PMTs for faster detection of FP signal and has a modular design with assay-specific cartridges for best performance. It supports up to 1536-well microplate formats and is HTS compatible with the optional StakMax® Microplate Stacker. With optimized settings for the Transcreener FP assays, the SpectraMax Paradigm Microplate Reader exceeds validation requirements.

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 acquisition. Proper selection of instrument settings have a profound impact on the sensitivity of the assays. The key instrument parameters for Transcreener HTS assay performance were determined by running a standard curve for conversion of 10 µM ATP to ADP, mimicking a typical kinase enzyme reaction. Starting with 10 µM ATP, ADP was added in increasing amounts and ATP was decreased proportionately, maintaining a total adenine nucleotide concentration of 10 µM. The integration times were varied to determine the requirements for a Z' > 0.5. Validation of an instrument for use with the Transcreener FP Assays requires a Z' of at least 0.7 and a Δ mP greater than 120 at 10% conversion of 10 µM ATP (1 µM ADP/9 µM ATP).

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 ADP^2 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 ADP^2 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).

Methods

Assay preparation

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.

Instrument setup

Set up the SpectraMax Microplate Reader with the settings in Table 1.

Table 1. Recommended SpectraMax Paradigm Instrument Settings

Parameter	Setting
Detection cartridge	Fluorescence Polarization
Filters: Wavelength/bandwidth	Excitation: 624/40, Emission: 684/24
Read type	Endpoint
PMT and Optics	Stop and Go with 20 ms or higher integration time, or on-the-fly performance
Read height	6.61 mm for Corning 3676 plate; optimize for other plates

The SpectraMax Paradigm plate reader is operated using SoftMax® Pro Software. Start with a pre-configured protocol from the protocol library and modify as described.

1. Open the SoftMax Pro Software and click on 'Protocol Manager', then 'Protocol Library', to access the pre-configured protocols. In the 'Paradigm Protocols' folder, select the 'FP Rhodamine' protocol.

2. Click 'Plate01' in the Navigation Tree, and click on Settings (gear icon) to open the Settings dialog.

3. Select the custom cartridge for Alexa Fluor 633 FP in the Cartridges menu.

4. Read Mode 'FP' and Read Type 'Endpoint' should be highlighted.

5. Wavelength settings are determined by the cartridge and are not user-changeable.

6. Click 'Plate Type' and choose Plate Format '384 Wells' and Select Specific '384 Well Corning low vol/rndbtm'.

7. Click 'Read Area' and choose the wells to read on the plate diagram.

8. Click 'PMT and Optics'. Select 'Off – Stop and Go' or 'Performance – On the fly'.

9. If Stop and Go is selected, enter an integration time of 20 ms or higher. Longer integration times typically offer better performance, but plate reading will take longer.

10. If optimal read height is known, enter it. For any new assay or plate type, read height optimization should be performed. If optimization will be performed, leave the read height at the 1 mm default value. After optimization is performed, the new (optimal) read height will replace the default value.

11. Click 'More Settings'. Choose a read order, 'Row' if a whole plate or a few complete rows will be read, or 'Column' if only a few complete columns will be read.

12. Check the box next to 'Show Pre-Read Optimization Options'.

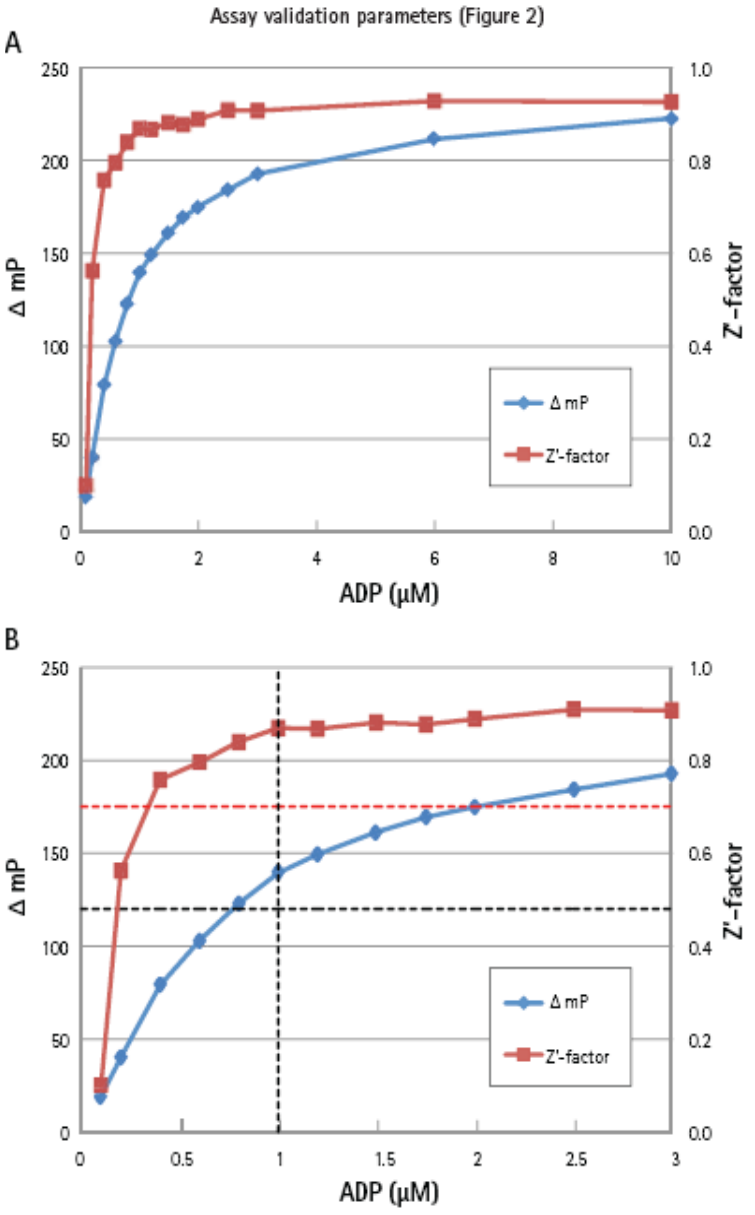
13. Click 'OK' to close the Settings dialog.

14. Click Read. A wizard showing optimization options will appear. Perform both microplate optimization and read height optimization for any new assay.

Results

Sample FP standard curve

As the ratio of ADP to ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in polarization values. Assay plates containing the 15-point standard curve were read on a SpectraMax Paradigm Microplate Reader from Molecular Devices.



A: Z' and Δ mP values observed in a standard curve for conversion of 10 µM ATP to ADP. **B**: Zoomed view of the 0-3 µM ADP section

of the standard curve, with dasehed lines indicating that the observed Z' and Δ mP values exceed the validation requirements. Plate reader set at 100 ms integration time.

Table 2. Assay performance with various instrument settings							
Assay performance, 10% conversion 10 µM ATP							
Integration time	20 ms	30 ms	50 ms	100 ms	150 ms	200 ms	OTF
Read Time (minutes)	1:02	1:08	1:17	1:42	1:59	2:17	0:55
Δ mP at 0% ATP conversion	147	146	144	139	136	151	148
Z'-Factor at 10% ATP conversion	0.77	0.80	0.79	0.87	0.84	0.91	0.77

Conclusions

This application protocol demonstrates the validation of the Molecular Devices SpectraMax Paradigm Microplate Reader for use with the Transcreener FP Assays. By utilizing the optimized instrument settings suggested here, Z' values > 0.7 and Δ mP > 120 are achievable with read times under 1 minute. Results greatly exceeding minimum validation requirements can be achieved by using longer integration times, while the faster on-the-fly setting meets assay requirements with a read time of less than one minute.

Additional Information

Please visit www.bellbrooklabs.com or contact BellBrook Labs for pricing for the Transcreener® Assays. Custom quotes are available for bulk orders.

Ordering Information

Phone Orders:
608.443.2400
866.3137881

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