

## Optimized Settings to Validate the Molecular Devices Analyst® GT/HT Microplate Readers with the Transcreener® Fluorescence Polarization Assays.

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*This Application Note describes the optimal instrument parameters used to validate the Analyst® GT/HT plate readers with the following assays:*

*Transcreener® ADP<sup>2</sup> 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.

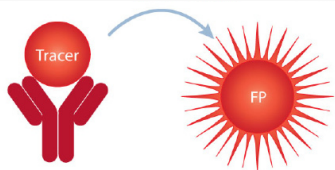


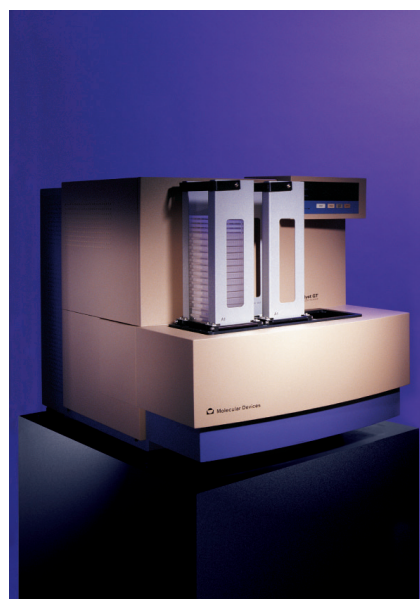
Figure 1. Transcreener® FP Assay Principle

### 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 number of 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  $\mu\text{M}$  ATP/ADP standard curve (24 replicates), as standard curves of this type mimic enzyme reactions. Starting with 10  $\mu\text{M}$  ATP, ADP was added in increasing amounts and ATP is decreased proportionately, maintaining a total adenine nucleotide concentration of 10  $\mu\text{M}$ . The integration times 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  $\Delta\text{mP} > 120$  at 10% conversion of 10  $\mu\text{M}$  ATP were required.

### Analyst® GT/HT Information

- Standard 25mm filters for FP, TRF, TR-FRET, FI, and Absorbance (separate PMT for Luminescence).
- Readout maximization powered by 2<sup>nd</sup> generation Smart Optics® system.
- HTS compatible, high-speed bidirectional stacker option.
- Seamless integration into robotic environments.
- Plate formats supported: 96- to 1536-well microplates.



### Instrument Settings

#### Instrument Wavelength Settings (Molecular Devices Catalog #)

EXC Wavelength/Bandwidth	620/30 nm (42-000-0038)
EMS Wavelength/Bandwidth	682/22 nm (XF3031 (Omega Optical))
Dichroic Mirror	650 nm (0200-6120)

#### Optimized Analyst® GT Settings

Lamp	Continuous
Z-Height	1 mm
Settle Time	10 ms (in advanced set up)
Attenuator Mode	Out
Raw Data Units	Counts/sec
Target SD/well	Not Set
Integration Time	Variable

Table 1. Recommended Analyst® GT Instrument Settings

## Instrument Setup

The AnalystHost application provides a graphical user interface for setting up the reader and running assays. Proceed with the following steps to optimize the instrument:

1. Open the AnalystHost software, and select "Detection" from the *Methods* menu. Then select "New".
2. Select "Fluorescence Polarization" from the dropdown list and input a name for the method.
3. Click "OK" to view the *Define and Edit Methods* screen. The new method will be assigned the default parameters for the selected method type.
4. Do not modify *Continuous Lamp*, *Raw Data Units of Counts/Sec* or *Attenuator Mode Out* from the default settings. *Continuous Lamp* is recommended for highest sensitivity in the fluorescence polarization detection mode.
5. Select the appropriate microplate format being used from the *Plate* dropdown menu.
6. Select "New" from the *Plates* menu, and input the plate name and number of wells. Click "OK" and input the microplate dimensions (in millimeters) in the microplate schematic. Click "OK" to save.

The same measurement settings can be used for subsequent plates as long as the volumes, tracer and concentrations remain the same.

## 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 Molecular Devices' Analyst® GT 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 B, 4 nM ADP Alexa633 Tracer, and 14.8 μg/mL ADP<sup>2</sup> 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<sup>2</sup> 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](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.

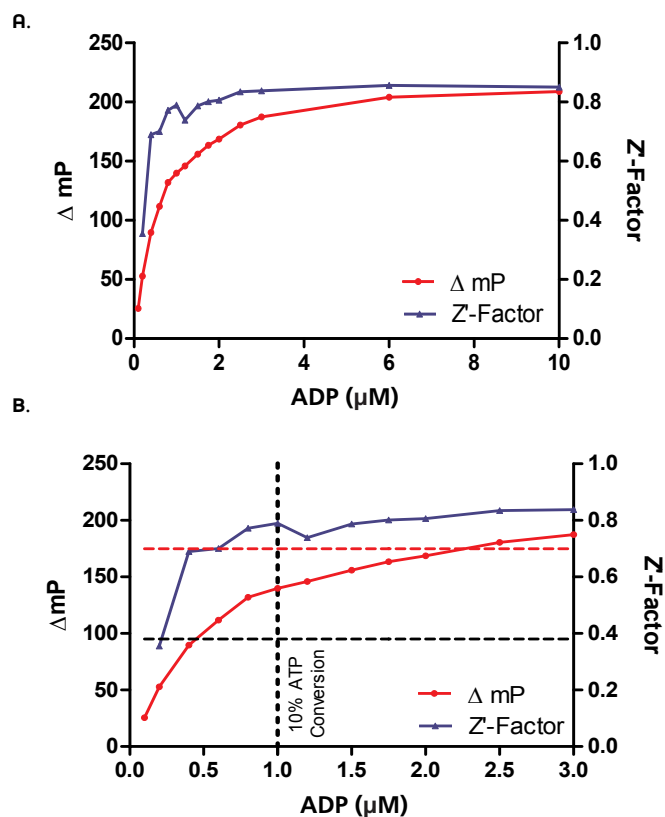


Figure 2. A). Z' and  $\Delta$  mP values observed in a standard curve mimic conversion of 10 μM ATP to ADP. B). Zoomed view of the 0-3 μM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and  $\Delta$  mP validation minimal qualification data (black dashed line). 10% ATP conversion validation point is indicated by the black dotted line. Plate reader set at 50,000 μs integration time.

Assay Performance, 10% Conversion 10 μM ATP					
Integration Time	30K	50K	75K	100K	150K
Read Time (minutes)	2:00	2:13	2:36	2:52	3:36
$\Delta$ mP at 10% ATP Conversion	140	140	138	133	137
Z'-Factor at 10% ATP Conversion	0.71	0.79	0.80	0.78	0.81

Table 2. Assay Performance with Various Instrument Settings

## Conclusions

This application note demonstrates the validation of the Molecular Devices Analyst® GT and Analyst® HT 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  $\Delta$ mP > 120 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.

Analyst® a registered trademark of Molecular Devices.

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

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.

