SpectraMax® Microplate Readers: A complete solution for Transcreener® assays

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Abstract

Transcreener® ADP2 Assays are homogenous assays with fluorescent readouts that enable the detection and screening of established drug targets including protein and lipid kinases, as well as emerging targets such as carbohydrate kinases, triphosphatases, heat shock proteins and other ATPases. The assay is based on the immunodetection of ADP. Three detection modes are offered to accommodate users' needs and detection format preferences: fluorescence polarization (FP), time-resolved Forster-resonance energy transfer (TR-FRET), and fluorescence intensity (FI).
Molecular Devices' SpectraMax® Microplate Readers have been validated for Transcreener ADP2assays and enable users to choose the detection format they prefer. The SpectraMax® Paradigm® Modular Multi-Mode Reader also offers user upgradeability so that new detection capabilities can be added as users' screening needs evolve.

Assays for screening in three detection modes

The Transcreener® ADP2 Assays measure activity of any enzyme that produces ADP. These assays have a homogeneous mix-and-read format and use far-red tracers to minimize interference from compounds and light scattering.

FP
In the FP assay, the Transcreener ADP Detection Mixture comprises an ADP Alexa633 Tracer bound to an ADP2 Antibody. The tracer is displaced by ADP, the invariant product generated during the enzyme reaction (Figure 1). The displaced tracer freely rotates leading to a decrease in fluorescence polarization.

The FP assay was performed using the SpectraMax® Paradigm® Modular Multi-Mode Microplate Reader.

TR-FRET
In the TR-FRET assay, 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 (about 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 (Figure 4). The time gated nature of the detection method largely eliminates interference that can result from prompt fluorescence of test compounds.

The TR-FRET assay was performed using the SpectraMax® M5 Multi-Mode Microplate Reader.

FI
In the fluorescence intensity assay, the Transcreener ADP Detection Mixture comprises a quenched ADP Alexa594 Tracer bound to the ADP2 monoclonal antibody conjugated to an IRDye® QC-1 quencher licensed from LI-COR®. 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 (Figure 6). Therefore, ADP production is proportional to an increase in fluorescence.

The FI assay was performed using the SpectraMax® M2 Multi-Mode Microplate Reader.

SpectraMax® Paradigm® Multi-Mode Microplate Reader

- Flexible design for future expansion
- User upgradable in < 2 minutes
- High speed detection for up to 1536-well microplates
- Excellent sensitivity and performance
- Future Ready

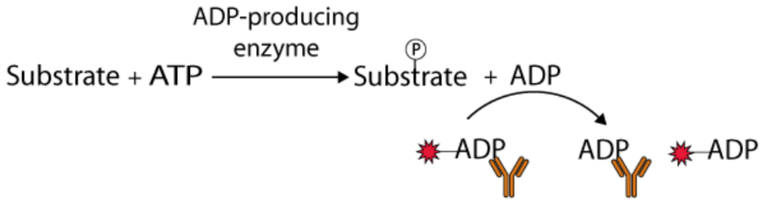


Figure 1. Transcreener ADP2 FP assay principle.

An assay plate containing a 15-point standard curve was read on the SpectraMax® Paradigm® Multi-Mode Microplate reader. As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in mP values. Minimum validation criteria are Z' > 0.7 and delta mP > 120 at 10% conversion.

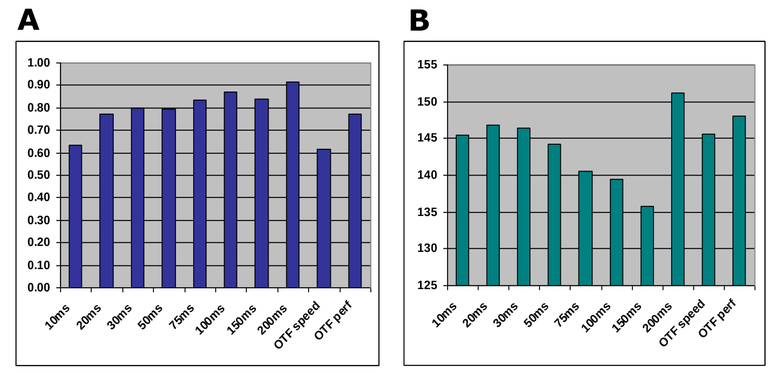


Figure 2. Standard curve results for FP assay. A) Z' values for different read settings. B) Assay window (delta mP) for different read settings.

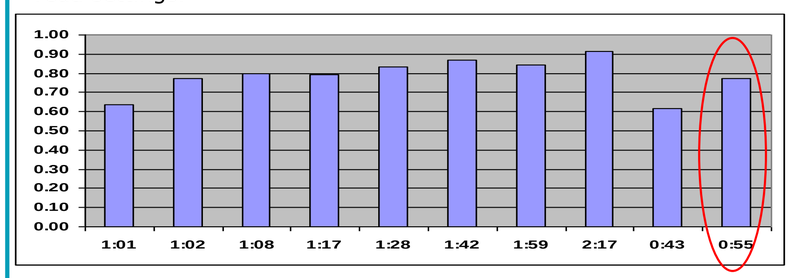


Figure 3. Z' values vs. read times. Results meeting validation criteria were achieved with a total read time of less than 1 minute.

SpectraMax® M5 Multi-Mode Microplate Reader

- Dual monochromator optics and 5 modes for a wide range of applications
- Patented AutoPMT Optimization System for plate-to-plate consistency of results and extended dynamic range
- SoftMax® Pro Software for complete data analysis

Transcreener® ADP2 TR-FRET assay

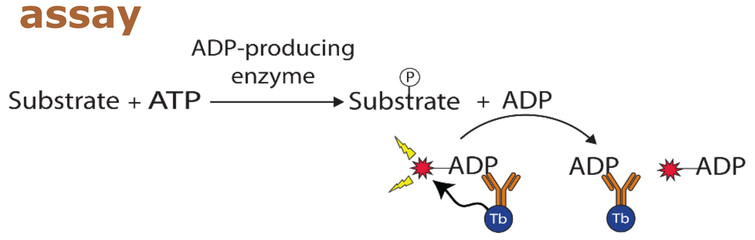


Figure 4: Transcreener ADP2 TR-FRET assay principle.

An assay plate containing a 15-point standard curve was read on the SpectraMax® M5 Microplate reader. As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in FRET.
Z' values were calculated at each ratio of ADP:ATP (Figure 5).

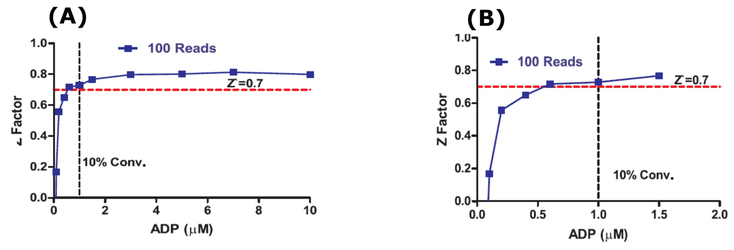
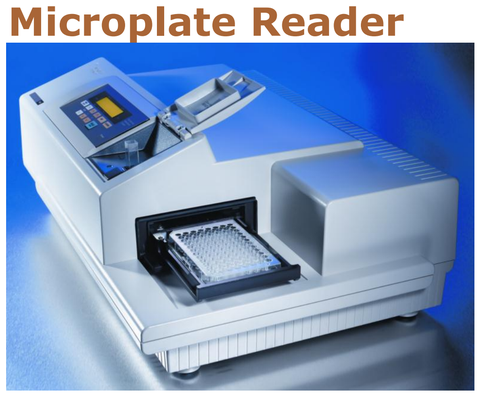


Figure 5. A) Z' values observed in a standard curve mimicking conversion of 10 µM ATP to ADP. B) A zoom in of the 1-2 µM ADP section of the standard curve. Z' validation minimal qualification shown by red dotted line. 10% ATP conversion validation point shown by black dotted line. Reader set at 100 reads per well.

Instrument Settings for SpectraMax® M5 Reader	
Excitation wavelength	320 nm
Emission wavelength 1	665 nm
Emission wavelength 2	620 nm
Read mode	Time Resolved Fluorescence
Delay	50 µsec
Integration	500 µsec
Read position	Top
Readings per well	100
PMT	Auto

Table 1. Instrument settings used with the SpectraMax® M5 Microplate Reader. 100 readings per well gives optimal Z' values for this assay.

SpectraMax® M2 Multi-Mode Microplate Reader



- Dual monochromator optics with 2 detection modes
- Patented AutoPMT Optimization System
- SoftMax® Pro Software for complete data analysis

Transcreener® ADP2 FI assay

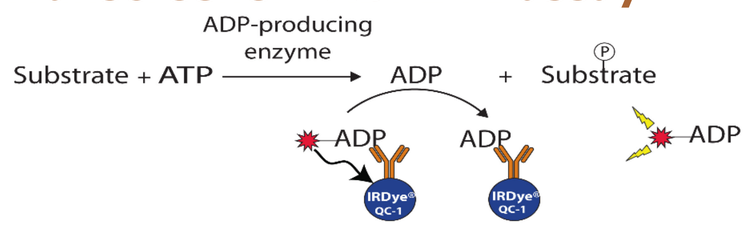


Figure 6: Transcreener ADP2 FI assay principle.

An assay plate containing a 15-point standard curve was read on the SpectraMax® M2 Microplate reader (Figure 1). As the ratio of ADP:ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall increase in RFU values.
Z' values were calculated at each ratio of ADP:ATP (Figure 7).

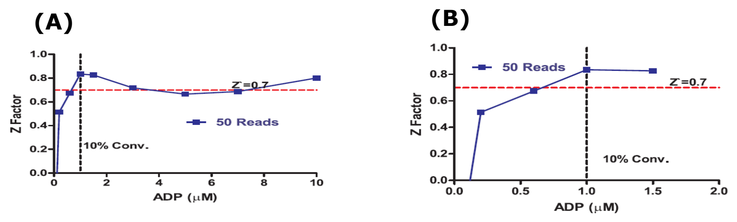


Figure 7. A) Z' values observed in a standard curve mimicking conversion of 10 µM ATP to ADP. B) A zoom in of the 1-2 µM ADP section of the standard curve. Z' validation minimal qualification shown by red dotted line. 10% ATP conversion validation point shown by black dotted line. Reader set at 50 reads.

Instrument Settings for SpectraMax® M5 Reader	
Excitation wavelength	575 nm
Emission wavelength	620 nm
Read mode	Fluorescence Intensity
Read position	Top
Readings per well	10-50
PMT	Auto

Table 2. Instrument settings used with the SpectraMax® M2 Microplate Reader. 50 readings per well gives optimal Z' values for this assay.

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