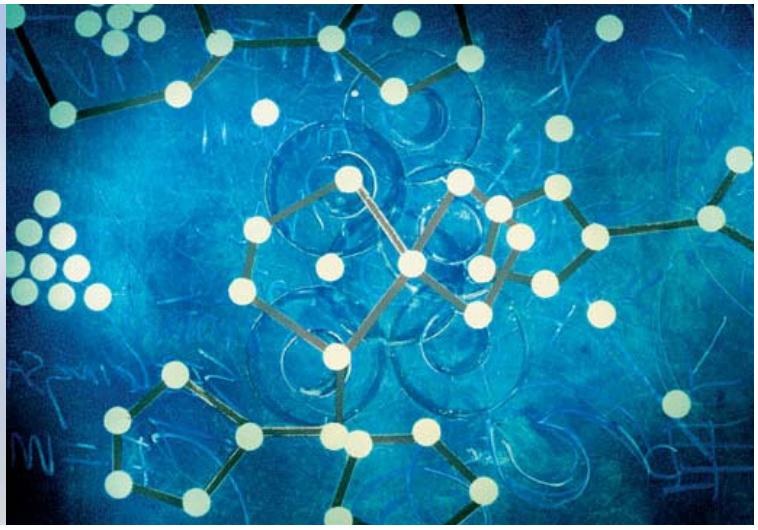
Transcreener® ADP² Fluorescence Intensity Assay

Implementation on Tecan's Infinite® 200 Multimode Reader Series



Introduction

The Transcreener ADP² fluorescence intensity assay

BellBrook Labs' Transcreener technology covers a number of universal, biochemical assays, based on the detection of nucleotides (i.e. ADP, AMP, GMP, GDP, UDP), which are formed by thousands of cellular enzymes, many of which catalyze the covalent regulatory reactions that are central to cell signaling and represent new opportunities for drug discovery.
The Transcreener ADP² Assay is new and offers greater sensitivity than the original Transcreener ADP Assay. The improvement is due to a more sensitive antibody against ADP. Greater sensitivity results in an excellent signal at less than 10% ATP consumption for a broad range of initial ATP concentrations (0.1-1000 µM). The result is the ability to screen low ATP K_m enzymes, and to use initial velocity enzyme kinetics at or below ATP K_m concentrations, which leads to accurate inhibitor potencies and the ability to use less enzyme and substrate (1).
Like its predecessor, the Transcreener ADP² Assay is a homogenous assay that enables the facile detection and screening of established drug targets including protein and lipid kinases as well as emerging targets such as heat shock proteins and other ATPases. The assay is based on the immunodetection of ADP and allows the screening of diverse enzymes with native and synthetic substrates, or enzymes with intrinsic ATPase.
The Transcreener ADP² Assay is available in three fluorescence detection formats: fluorescence polarization (FP), fluorescence intensity (FI) and time resolved fluorescence resonance energy transfer (TR-FRET). The dye used for all three assays emit in the red region of the visible spectrum to minimize compound interference.

The Transcreener ADP² FI Assay extends the Transcreener platform for ADP detection by utilizing a simple fluorescent intensity output which can be used on fluorescence readers typically found in academic and therapeutic research labs as well as more complex multimode plate readers more commonly used in core facilities and HTS laboratories. The Transcreener ADP² FI Assay is a red, competitive fluorescence intensity assay based on the detection of ADP and is therefore compatible with any enzyme class that produces ADP, including protein, lipid, and carbohydrate kinases, ATPases, DNA helicases, carboxylases and glutamine synthetase. It is a simple, one step, homogenous detection assay, and is flexible with regard to ATP concentration (0.1 to 1000 µM ATP) (2).

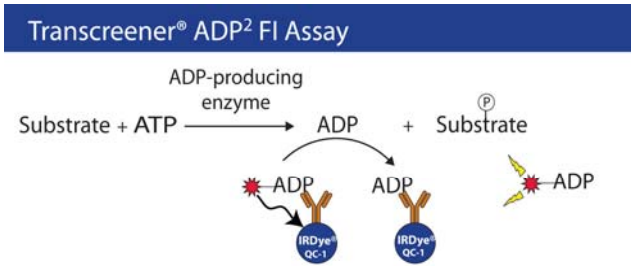


Figure 1: Transcreener ADP^2 FI assay principle (1).

The Transcreener ADP^2 FI Assay was developed to follow the progress of any enzyme that produces ADP. The *Transcreener ADP Detection Mixture* comprises quenched *ADP Alexa594 Tracer* bound *ADP^2 Antibody conjugated to 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 an increase in fluorescence intensity (2).

This technical note describes the successful implementation of the Transcreener™ Fluorescence Intensity Assay on Tecan's quad-4™ monochromator-based Infinite M200 and Tecan's filter-based Infinite F200 multimode reader.

Material and methods

Instrument

- Infinite F200 filter-based multimode reader
- Infinite M200 quad4 monochromator-based multimode reader

Microplates

384 well, flat bottom, black, polystyrol, small volume (Greiner®, Germany)

Reagents

Transceener ADP^2 FI Assay, # 3013-K (BellBrook Labs, Madison, WI, USA)

Assay procedure

According to the Transcreener FI technical manual (V073009) several 14-point curves using ATP and ADP concentration corresponding to the following ATP conversions were generated: 0, 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 30, 40, 60, 100 %. The following initial ATP concentrations (as 0% conversion) were used: 0.1 µM, 1 µM, 10 µM and 100 µM ATP.

ADP (nM)	ATP (nM)	ATP Conversion (%)
0	10000	0
50	9950	0.5
100	9900	1
200	9800	2
400	9600	4
600	9400	6
800	9200	8
1000	9000	10
1500	8500	15
2000	8000	20
3000	7000	30
4000	6000	40
6000	4000	60
10000	0	100

Table 1: ATP/ADP dilution series and corresponding ATP conversions exemplified for an initial ATP concentration of 10 µM.

Reagent addition and data reduction was performed according to the Transcreener FI technical manual (2).

Measurement parameter and Instrument settings

Measurement paramter	Instrument setting
Plate	GRE384sb.pdfx
Mode	Fluorescence Intensity top
Excitation wavelength	585 nm
Excitation bandwidth	9 nm
Emission wavelength	627 nm
Emission bandwidth	20 nm
Gain	optimal
Number of flashes	25 - 100
Settle time	0 msec
Lag time	0 µs
Integration time	20 µs

Table 2: Measurement parameter and instrument settings of the Infinite M200.

Measurement	Instrument setting
Plate	GRE384sb.pdfx
Mode	Fluorescence Intensity top
Excitation wavelength	580 nm
Excitation bandwidth	20 nm
Emission wavelength	620 nm
Emission bandwidth	20 nm
Gain	optimal
Number of flashes	25 - 100
Settle time	0 msec
Lag time	0 µs
Integration time	20 µs

Table 3: Measurement parameter and instrument settings of the Infinite F200.

Results

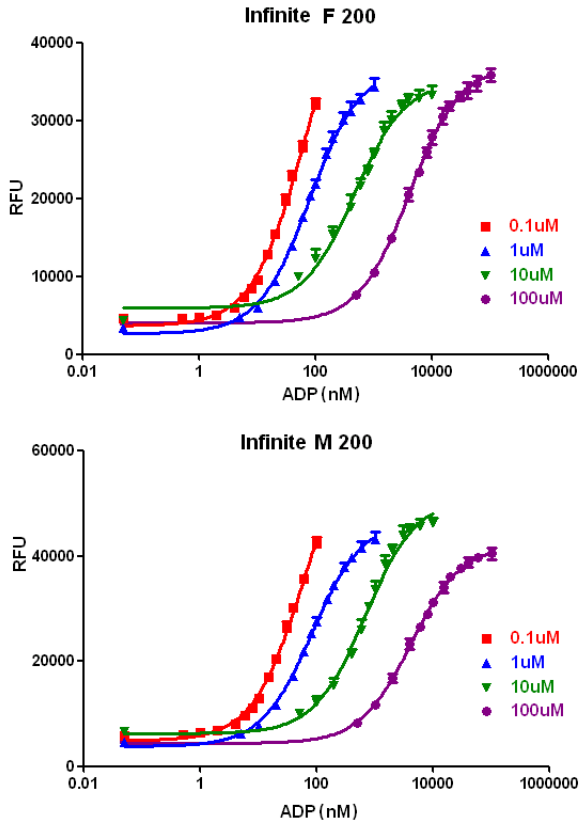


Figure 2: Sample data for 0.1 µM, 1 µM, 10 µM, and 100 µM ADP/ATP standard curves generated with a flash number of 100 for both instruments.
The nucleotide concentration reflects the amount in the enzyme reaction prior to the addition of the ADP Detection Mixture. Data are plotted as RFU vs log [ADP] using nonlinear regression curve fitting.

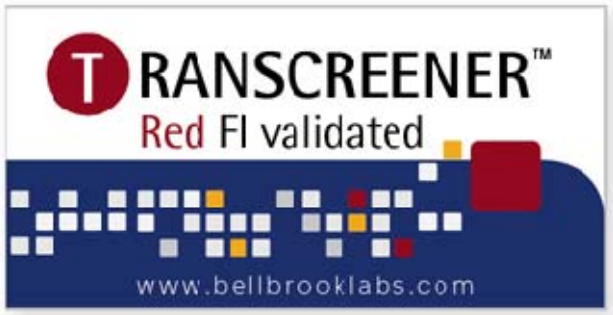
	Infinite F200				Infinite M200			
	0.1µM ATP	1µM ATP	10µM ATP	100µM ATP	0.1 µM ATP	1µM ATP	10µM ATP	100µM ATP
CV%	3.24	2.49	3.15	3.0	2.84	3.04	4.62	2.10
Z´	0.67	0.87	0.87	0.88	0.74	0.87	0.80	0.91

Table 4: CV% and Z´values at 10% ATP conversion for the different dilution curves; data correspond to measurements using 100 flashes.

Figure 2 and table 4 clearly show the outstanding performance of the Infinite M200 and the Infinite F200 when measuring the Transcreener FI assay and using 100 flashes per well. The CVs as well as the Z´ values at 10 % ATP conversion are excellent for all tested ADP/ATP standard curves. But even with a flash number of only 25 flashes per well the results were very good and within the criteria given by BellBrook Labs. For instance the Z´value at 10% ATP conversion using 10 µM ATP as initial concentration was above 0.7 for both instruments (i.e. 0.77 for the Infinite M200 and 0.86 for the Infinite F200) when using only 25 flashes. Due to this fact, the Infinite 200 series in combination with the Transcreener FI assay may even be used in medium-throughput screening applications where measurement time has a critical impact.

Conclusion

The data presented in this technical note clearly demonstrates the compatibility of Bellbrook Lab's Transcreener FI assay with Tecan's Infinite M200 and Infinite F200, respectively. In combination, BellBrook Labs Transcreener FI assay and Tecan's Infinite 200 series provide an affordable but highly sensitive system to study enzyme activity in an academic and/or therapeutic context.



Literature

[1] www.bellbrooklabs.com
[2] Transcreener® ADP² FI assay, Technical Manual V073009 (BellBrook Labs, Madison, WI, USA)

List of Abbreviations

ADP	adenosine diphosphate
ATP	adenosine triphosphate
FI	fluorescence intensitiy
CV	correlation value

Acknowledgement

We express our acknowledgements to **Meera Kumar** and **Karen Kleman-Leyer** from BellBrook Labs, LLC, 5500 Nobel Drive, Suite 250 Madison, WI 53711, USA, who provided excellent support for the validation of the Infinite F200 and Infinite M200.

Austria T +43 62 46 89 33 **Belgium** T +32 15 42 13 19 **China** T +32 15 42 13 19 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80
Germany +49 79 51 94 170 **Italy** +39 02 215 21 28 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Portugal** +351 21 000 82 16
Singapore +65 644 41 886 **Spain** +34 93 490 01 74 **Sweden** +46 31 75 44 000 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300
USA +1 919 361 5200 **ROW** +43 62 46 89 33
www.tecan.com

396 125 V.1.0, 11-2009