

## Transcreener<sup>®</sup> ADP Assay – Far Red FP

### Implementation on Tecan's Infinite<sup>®</sup> F500 Multimode Reader



## Introduction

Transcreener<sup>®</sup> Technology is a universal, high throughput biochemical assay 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 represent new opportunities for drug discovery. Transcreener<sup>®</sup> Assays use far red fluorescence polarization (FP) which overcomes compound interference and is a simple and robust ratiometric detection method (2).

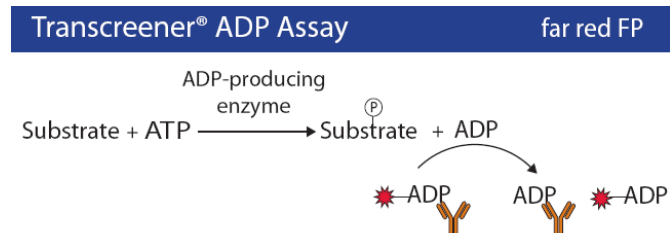
The Transcreener<sup>®</sup> ADP Assay is a competitive FP-assay based on the detection of ADP and therefore is compatible with any enzyme class that produces ADP, including protein-, lipid-, and carbohydrate-kinases, ATPases, DNA helicases, carboxylases- and glutamine synthetase (1).

This technical note describes the successful implementation of the Transcreener<sup>®</sup> Assay Technology on Tecan's Infinite F500 filter-based multimode reader.

### Assay principle

The Transcreener<sup>®</sup> ADP Assay is a simple one-step homogenous endpoint assay. The Transcreener<sup>®</sup> ADP Detection Mixture, comprised of an ADP Alexa633 Tracer bound to an ADP Antibody, is added to the enzyme reaction mix.

ADP, the invariant product generated during the enzyme reaction, displaces the tracer. The displaced tracer freely rotates leading to a decrease in fluorescence polarization. Therefore, ADP production and enzyme activity result in a decrease in polarization values (Figure 1) (1,2).



**Figure 1:** Principle of the Transcreener<sup>®</sup> ADP Assay.

## Material and Methods

### Instrument

Tecan Infinite F500 filter-based multimode microplate reader including a fluorescence polarization detection module

### Microplates

384 well micro plate, flat bottom, black, polystyrol (Corning®, NY)

### Reagents

Transcreener® ADP Assay (BellBrook Labs, Madison, WI, USA, Cat. No. 3004-1K)

### Assay procedure

The following experimental setup was used to validate the Infinite F500 for Transcreener® ADP assay:

A dilution series of ADP/ATP was prepared as summarized in Table 1.

ADP ( $\mu$ M)	ATP ( $\mu$ M)	ATP Conversion (%)
0	10	0
0.1	9.9	1
0.2	9.8	2
0.4	9.6	4
0.6	9.4	6
0.8	9.2	8
1	9	10
1.2	8.8	12
1.5	8.5	15
1.75	8.25	17.5
2	8	20
2.5	7.5	25
3	7	30
6	4	60
10	0	100

**Table 1:** ATP/ADP dilution series and corresponding %ATP conversions

10  $\mu$ l of each ATP/ADP combination was added to an equal volume of Transcreener® ADP Detection Mixture in a black 384 well plate and incubated for 1 hr at RT before being measured in fluorescence polarization mode on the Infinite F500.

To validate the instruments performance at different measurement speeds the flash number was continuously increased.

### Measurement parameter and Instrument settings

Measurement Parameter	Instrument Settings
Plate	COS384fb.pdf
Mode	Fluorescence Polarization
Excitation Wavelength	610 nm
Excitation Bandwidth	20 nm
Emission Wavelength	670 nm
Emission Bandwidth	25 nm
Gain (optimal)	94
Number of Flashes	3 - 20
Settle Time	0 msec
Z-Pos. (calc. from: A1)	22969
G-Factor (calculated)	0.842

**Table 2:** Measurement parameters and instrument settings of the Infinite F500 for the Transcreener® ADP Assay

## Results

By varying the flash number and leaving detector gain, beam focus (z-position) and G-factor at the instruments optimized settings, the standard Transcreener® ADP Assay Kit enabled robust detection of  $\leq 10\%$  ATP conversion in the exemplified 3-20 flashes range. As shown in Table 3 the optimal relation between measurement speed and instrument performance is achieved with 5 flashes.

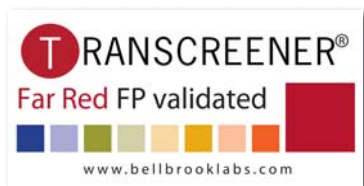
Even with only 3 flashes per well and a corresponding measurement time of only 2.5 min for a 384 well plate the Z'-Factor was above 0.7 which clearly demonstrates an excellent performance of Tecan's Infinite F500 filter-based multimode microplate reader for the described assay format.

	No. of flashes			
	3	5	10	20
$\Delta$ mP	108	109	108	109
SD.	4.7	4.5	4.5	4.6
Z' - Factor	0.74	0.78	0.76	0.76
Read time [min:sec]	2:27	3:10	5:47	8:07

**Table 3:** Effect of flash number on read time and data quality at 10 % ATP conversion. The data shown are exemplified **typical performance values** achieved during the validation at BellBrook Labs.

## Conclusion

Tecan's filter-based multimode microplate reader Infinite F500 has been successfully **Transcreener® Far Red FP validated** by BellBrook Labs, LLC, USA.



## List of Abbreviations

ADP	adenosine diphosphate
ATP	adenosine triphosphate
FP	fluorescence polarization
SD	standard deviation

## Acknowledgement

We express our acknowledgements to Brad Larson from **BellBrook Labs, LLC**, 5500 Nobel Drive, Suite 250 Madison, WI 53711, USA, who provided excellent support for the validation of the Infinite F500.

## Literature

- [1] Transcreener® ADP Assay, Technical Manual (BellBrook Labs, Madison WI, USA); modified
- [2] www.bellbrooklabs.com

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