Transcreener® ADP² TR-FRET Red Assay

Implementation on Tecan's Infinite® multimode reader series

Introduction

Adenosine diphosphate (ADP) is an important mediator of cellular metabolism that is essential for maintaining energy levels. The Transcreener ADP² TR-FRET Red Assay is intended for the detection of ADP production by any kinase or ATPase, and has been developed specifically for high throughput screening applications. The assay is based on the competitive binding of ADP to a monoclonal antibody-terbium conjugate, and uses a homogeneous TR-FRET detection mechanism (1). In the absence of free ADP, excitation of terbium results in energy transfer to a far-red tracer and emission at 665 nm. Any ADP produced by the enzyme of interest displaces the tracer from the antibody, disrupting the FRET reaction (see Figure 1).

The Infinite F200 PRO, Infinite F500 and Infinite M1000 PRO multimode readers were tested for their capacity to measure the Transcreener ADP² TR-FRET Red Assay.

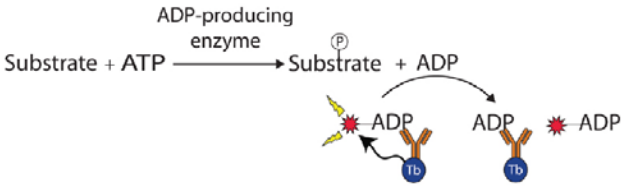


Figure 1 Principle of the Transcreener ADP² TR-FRET Red Assay (2).

Materials and methods

- Infinite F200 PRO filter-based multimode microplate reader
- Infinite F500 filter-based multimode microplate reader
- Infinite M1000 PRO premium Quad4 Monochromators™-based multimode microplate reader
- Transcreener ADP^2 TR-FRET Red Assay (BellBrook Labs, USA)
- 384-well white, low volume microplates (Greiner®, Germany)

Following the assay technical manual (2), dilution curves were created at several ATP concentrations (1, 10 and 100 µM), corresponding to ATP conversions of 0, 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 30, 40, 60 and 100 %.

Reagent addition and data reduction were performed according to the assay technical manual (2), using a final filling volume of 20 µl. Measurements were performed as described in Tables 1-3.

Measurement parameter	Donor settings	Acceptor settings
Plates	[GRE384sw.pdfx]	[GRE384sw.pdfx]
Mode	Fluorescence intensity top	Fluorescence intensity top
Excitation wavelength	340 nm	340 nm
Excitation bandwidth	35 nm	35 nm
Emission wavelength	620 nm	665 nm
Emission bandwidth	10 nm	8 nm
Gain	Optimal	Optimal
Number of flashes	25	25
Mirror	Automatic	Automatic
Integration time	500 µs	500 µs
Lag time	150 µs	150 µs
Settle time	0 ms	0 ms

Table 1 Measurement parameters and instrument settings for the Infinite F200 PRO.

Measurement parameter	Donor settings	Acceptor settings
Plates	[GRE384sw.pdfx]	[GRE384sw.pdfx]
Mode	Fluorescence intensity top	Fluorescence intensity top
Excitation wavelength	340 nm	340 nm
Excitation bandwidth	35 nm	35 nm
Emission wavelength	620 nm	665 nm
Emission bandwidth	10 nm	8 nm
Gain	Optimal	Optimal
Number of flashes	10	10
Mirror	Automatic	Automatic
Z-position	Calculated from well	Calculated from well
Integration time	500 µs	500 µs
Lag time	150 µs	150 µs
Settle time	0 ms	0 ms

Table 2 Measurement parameters and instrument settings for the Infinite F500.

Measurement parameter	Donor settings	Acceptor settings
Plates	[GRE384sw.pdfx]	[GRE384sw.pdfx]
Mode	Fluorescence intensity top	Fluorescence intensity top
Excitation wavelength	332 nm	332 nm
Excitation bandwidth	20 nm	20 nm
Emission wavelength	620 nm	665 nm
Emission bandwidth	50 nm	50 nm
Gain	Optimal	Optimal
Number of flashes	25	25
Flash frequency	100 Hz	100 Hz
Z-position	Calculated from well	Calculated from well
Integration time	500 µs	500 µs
Lag time	60 µs	60 µs
Settle time	0 ms	0 ms

Table 3 Measurement parameters and instrument settings for the Infinite M1000 PRO.

Results and discussion

As shown in Figure 2 and Table 4, all of Tecan's TR-FRET compatible multimode readers easily passed the validation criteria of the Transcreener TR-FRET ADP^2 Red Assay (criteria: Z' ≥ 0.7 for 10 % conversion using 10 µM ATP). The Z´ values at 10 % ATP conversion were excellent for all ATP/ADP standard curves tested.

	1 µM	10 µM	100 µM
Infinite F200 PRO	0.88	0.77	0.72
Infinite F500	0.92	0.84	0.78
Infinite M1000 PRO	0.92	0.88	0.72

Table 4 Z' values at 10 % conversion for different ATP/ADP standard curves.

All readers showed very good overall performance. With a measurement time of approximately 9 min for a whole 384-well plate, the Infinite F200 PRO reader is an excellent match for the requirements of academia and other low throughput screening applications. For medium throughput activities, such as assay development, the Infinite M1000 PRO combines tremendous flexibility and measurement times of approximately 7 min for an entire 384-well plate. The Infinite F500 offers the best performance/speed ratio – with measurement times of below 4 min for a whole 384-well plate – making it ideally suited to the requirements of high throughput screening applications.

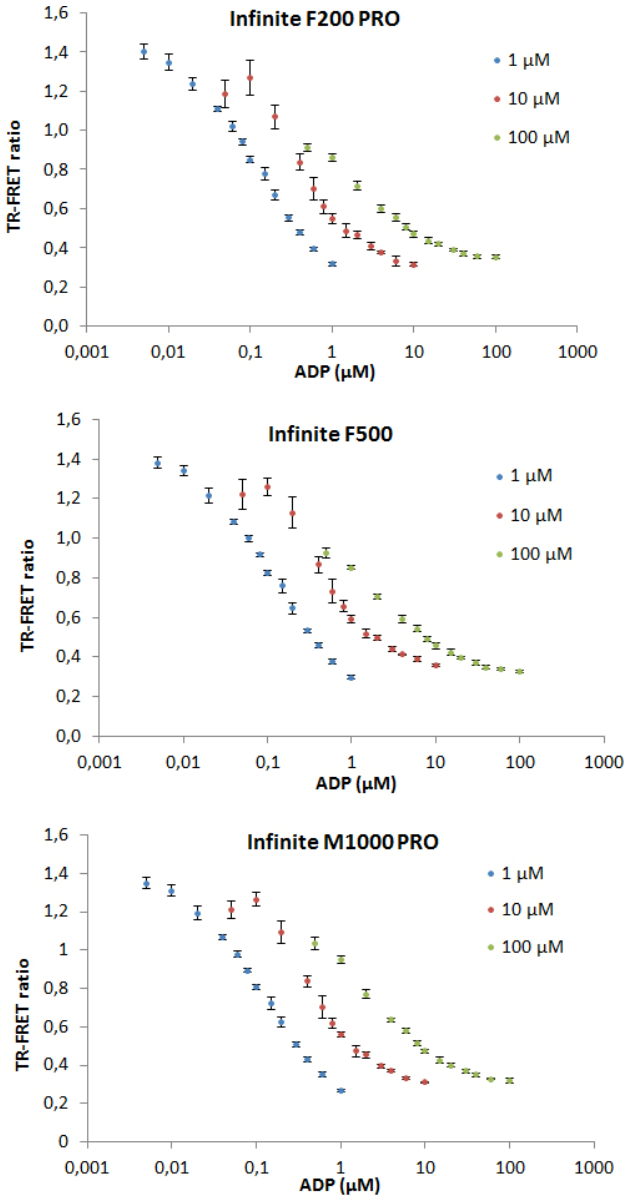
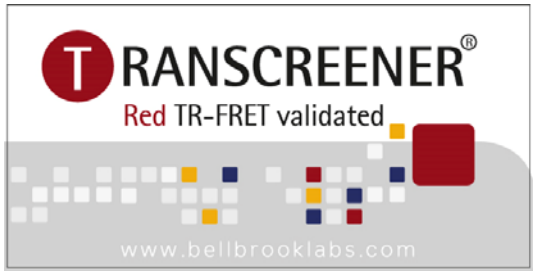


Figure 2 Sample data for 1, 10 and 100 µM ATP/ADP standard curves. Data is shown as TR-FRET ratios (665/620) vs log [ADP].

Conclusion

The data presented in this application note clearly demonstrates the compatibility of BellBrook Labs' Transcreener TR-FRET Red Assay with Tecan's Infinite series multimode readers, providing a highly sensitive system to study enzyme activity in an academic context, as well as for screening applications.



Literature

(1) www.bellbrooklabs.com
(2) Transcreener ADP^2 TR-FRET Red Assay, Technical Manual v071613 (BellBrook Labs, Madison, WI, USA)

List of abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
TR-FRET	Time-resolved fluorescence resonance energy transfer

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Australia +61 3 9647 4100 **Austria** +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 21 2206 3206 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80
Germany +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Singapore** +65 644 41 886 **Spain** +34 935 95 25 31
Sweden +46 31 75 44 000 **Switzerland** +41 44 922 81 11 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +43 62 46 89 33

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