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Optimizing Performance of Transcreener Fluorescence Polarization Assays with the Feyond-A500 Multi-Mode Microplate Reader

Transcreener technology is a universal, high-throughput biochemical assay platform based on nucleotide detection.

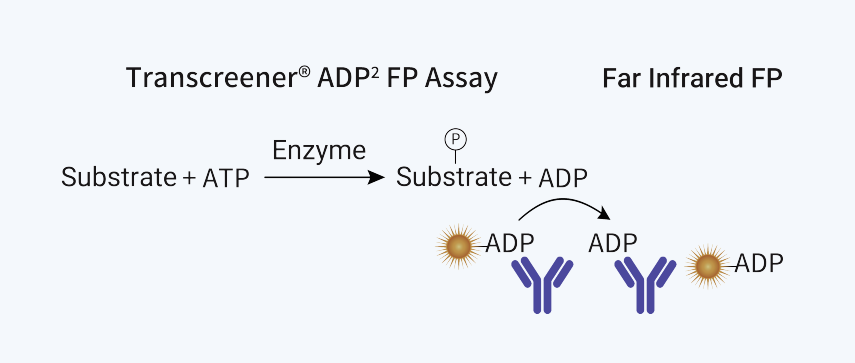
The assay is based on the detection of nucleotide diphosphates (ADP / GDP).

Nucleotide diphosphates are formed by thousands of kinases, many of which catalyze covalently regulated reactions.

These reactions are central to cellular signaling and are of great value in drug discovery.

Principle

The reagents for all the assays are a far-red tracer bound to a highly specific monoclonal / polyclonal antibody. An enzymatic reaction generates diphosphates or monophosphates, which displace the tracer from the antibody-quencher conjugate. This results in the generation of a signal due to an increase in rotational freedom of the tracer, detected as a decrease in polarization.



Verification Standards

Prepare a 10 μM ATP / ADP standard curve to simulate the enzyme reaction. Starting with 10 μM ATP, increase the amount of ADP added and decrease ATP proportionally, maintaining the total adenine nucleotide concentration at 10 μM. At a 10% conversion rate of 10 μM ATP, Z' > 0.7 and ΔmP > 120.

Materials and Methods

- Feyond-A500 Multi-Mode Microplate Reader (Hangzhou Allsheng)
- Transcreener® ADP² FP Assay (Code: No.3010-1K)
- ATP / ADP Mixture In Buffer (Constant Adenine Concentration: 10 μM)
- Corning 96-Well Black Polystyrene Plate (Code: No. 3915)

Concentration (%)	ATP	ADP
100	0	100
75	25	75
50	50	50
25	75	25
15	85	15
10	90	10
7.5	92.5	7.5
5	95	5
3	97	3
2	98	2
1	99	1
0	100	0

Table 1 ADP / ATP Standard Curve Preparation (10 μM)

Parameter	Fluorescence Polarization
EX	624-40 nm
EM	692-40 nm
G-factor	1.06
Number	150
PMT gain	Auto
Integration time	40 μs

Table 2 Instrument Parameter Settings

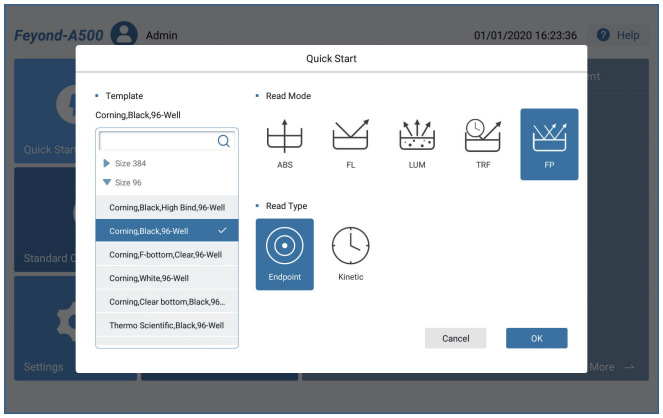


Figure 1 FP Measurement Mode Selection

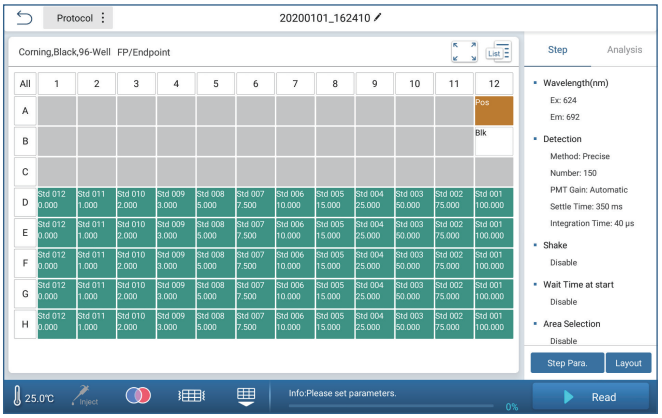
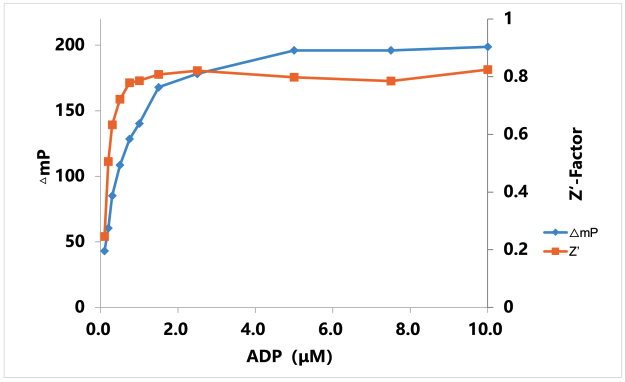


Figure 2 Instrument Parameter Settings

Results

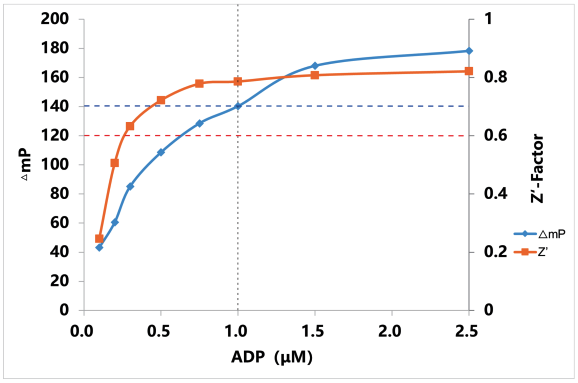
As the ratio of ADP to ATP increases, the proportion of bound tracer vs. free tracer decreases, resulting in an overall decrease in mP values.

A



A: Z' and ΔmP values observed in a standard curve mimic the conversion of 10 μM ATP to ADP.

B



B: Zoomed view of the 0-2.5 μM ADP section of the standard curve shows the Z' validation minimal qualification data (blue dashed line) and Δ mP validation minimal qualification data (red dashed line). The 10% ATP conversion validation point is also indicated (vertical black dotted line).

Conclusion

The Feyond-A500 Multi-Mode Microplate Reader passed the validation criteria for the Transcreener ADP^2 FP assay. The filter-based measurement results showed a Z' value of 0.79 (standard: Z' value > 0.70 at 10% conversion at 10 μM ATP) and a Δ mP of 140 (standard: ΔmP > 120 mP at 10% conversion at 10 μM ATP).

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