

New Transcreener® ADP² FP Assay performed on BMG LABTECH's PHERAstar *Plus* HTS Microplate Reader



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Introduction

BellBrook Labs has developed the universal Transcreener® ADP² Assay, a homogeneous, competitive fluorescent polarization HTS assay that directly detects ADP, the invariant reaction product of all kinase reactions, as well as heat shock proteins and other ATPases. These enzymes catalyze the covalent regulatory reactions that are central to cell signaling and are high value targets in drug discovery.

The Transcreener ADP² FP Assay is a new assay, with greater sensitivity than the original Transcreener ADP Assay.¹ The improvement is a more sensitive antibody against ADP yielding an excellent signal at less than or equal to 10 % ATP consumption for a broad range of initial ATP concentrations (0.1-1,000 μ M).

BMG LABTECH's PHERAstar *Plus* is a multi-detection microplate reader that combines rapid plate reading necessary for HTS with enhanced performance and sensitivity needed to measure small liquid volumes. The PHERAstar *Plus* reads all HTS detection modes (fluorescence intensity, time-resolved fluorescence, fluorescence polarization, luminescence, and absorbance) in all plate formats up to 1536 wells.

The PHERAstar *Plus* uses a unique application-specific module in conjunction with an optical reading head featuring two matched pairs of photomultiplier tubes (PMTs) that can simultaneously measure two emission signals at any desired wavelength. FP, TR-FRET and BRET are powerful detection modes that benefit from the Simultaneous Dual Emission technology. The outstanding sensitivity of the PHERAstar *Plus* is based on a new, innovative lens-based optical design. This design provides for outstanding sensitivity

and accuracy, along with minimal read times. For FP assays, capturing both the parallel and perpendicular channels with one read dramatically decreases the inherent variability in reading the same plate twice.

Assay Principle

The Transcreener® ADP² Assay is a fluorescence polarization immunoassay based on the detection of ADP by an antibody (Figure 1). This assay platform provides the possibility to universally interrogate all enzymes that catalyze group transfer reactions with ATP. In step one of the assay, enzymes catalyze the transfer of phosphate from ATP to a protein, peptide, lipid or small molecule resulting in the accumulation of ADP.

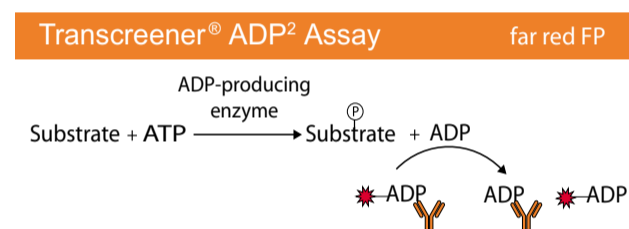


Fig. 1: Transcreener ADP² Assay Principle for Kinases

In step two the Transcreener® ADP² Detection Mixture, which contains an ADP Alexa633 tracer bound to an anti-ADP antibody, is added. If there is enzymatic activity resulting in necessary ADP then the bound tracer is displaced by the ADP. The free tracer rotates quickly leading to a lower polarization value. If there is no free ADP because of no enzymatic activity, the tracer is still bound to the antibody. This whole construct rotates very slowly giving a higher polarization number. Therefore, ADP production leads to a decrease in fluorescence polarization.

Materials and Methods

- Black 384 well and 1536 well microplates from Corning (#3676 and #3728)
- Transcreener® ADP² Assay from BellBrook Labs, Madison, WI, Cat. No. 3010-1K (including ADP Alexa633 Tracer, ADP² Antibody, Stop & Detect Buffer B, ATP, and ADP)
- PHERAstar *Plus*, BMG LABTECH, Offenburg, Germany

Using 10 μ M ADP and 10 μ M ATP stock solutions a 12 point ADP/ATP standard curve was prepared, while keeping a constant concentration of total adenosine.

This standard curve mimics a kinase or ATPase reaction (Note ADP is produced while ATP is depleted). The upper limit of the standard curve was set to 0 μ M ADP/10 μ M ATP (mimicking 0% conversion) and the lower limit was set to 10 μ M ADP/0 μ M ATP (mimicking

100% conversion). To the different ADP/ATP solutions the same volume of ADP Detection Mixture was pipetted. The antibody concentration was 7.4 μ g/mL. (For ideal assay performance it is important to determine an optimal antibody concentration under the specific enzyme and buffer conditions used in your experiment).²

The concentration of the far-red tracer was 2 nM. The solutions (384 well final volume 20 μ L, 1536 well final volume 8 μ L) were mixed and incubated for 1 hour at room temperature. The fluorescence polarization measurements were performed using the Transcreener® specific FP optical module with Excitation at 590 nm and Emission A (parallel) and Emission B (perpendicular) at 675 nm. The mP target was set to 20 mP for the free tracer.

Results

Figure 2 and 3 show the standard curves measured on the PHERAstar *Plus* in 384 well and 1536 well format, respectively. Graphing on the log scale eliminates the point that corresponds to zero. To include all twelve points along the curve, the value for 0 μ M ADP/10 μ M ATP was graphed at 0.01 μ M position.

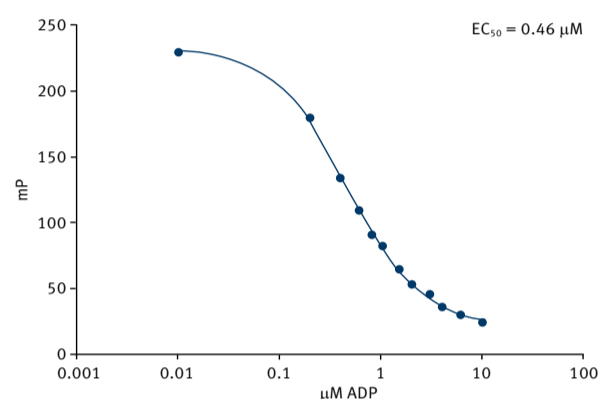


Fig. 2: ATP/ADP standard curve performed in a 384 well microplate

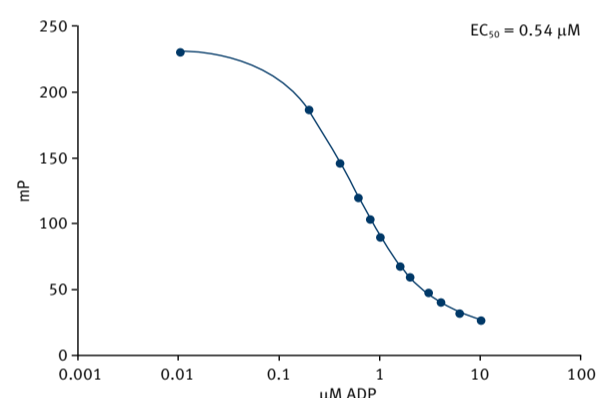


Fig. 3: ATP/ADP standard curve performed in a 1536 well microplate

Both graphs show similar assay windows and also similar EC₅₀ values indicating that the Transcreener® ADP² assay can be performed on the PHERAstar *Plus* using both plate types.

In order to show that the new Transcreener® ADP² assay is favorably compared to the former Transcreener® ADP assay, a Z' and Δ mP comparison between both assays was performed (Figure 4).

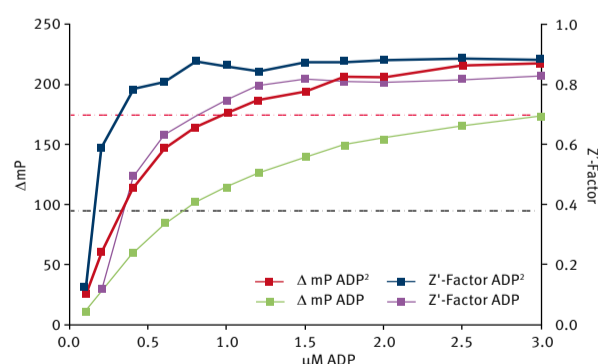


Fig. 4: Z' and Δ mP comparison between the Transcreener® ADP and ADP² FP assays. Z' validation minimal qualification is shown by the red dashed line. Δ mP validation minimal qualification is shown by the black dashed line.

The lowest % ATP conversion level yielding a Z' > 0.7 is obtained at 0.8 μ M ADP (or 8 % ATP conversion) for the ADP assay (purple line) whereas for the ADP² assay already at 0.4 μ M ADP (or 4 % ATP conversion) the intended Z' > 0.7 is reached (blue line).

Conclusion

The universally generic nature of the Transcreener® ADP² kit will reduce assay development efforts thus allowing HTS to occur earlier. As a characteristic parameter for the quality of the assay, a Z' value > 0.7 was calculated, which represents an excellent assay performance. Z' values between 0.5 and 1 indicate a highly robust screening assay and reflect high quality of instrumentation.³

The data show that the PHERAstar *Plus* (Figure 5) passes the validation criteria from BellBrook Labs.

The PHERAstar *Plus* microplate reader provides the ideal platform for the Transcreener® ADP² Assay. With its dual wavelength emission detection and five photomultiplier tubes (PMTs), the PHERAstar *Plus* provides the speed and sensitivity needed to take full advantage of BellBrook Labs Transcreener® technology. Furthermore, BMG LABTECH has designed an optic module specifically for BellBrook Labs' Transcreener®, thereby making assay setup simple.



Fig. 5: BMG LABTECH's multimode plate reader PHERAstar *Plus*

References

- 1 BMG LABTECH Application note 152: Transcreener® ADP Fluorescence Polarization Assay Performed on the PHERAstar
- 2 Transcreener® ADP² FP Assay Technical Manual, BellBrookLabs. Madison http://www.bellbrooklabs.com/PDFs/Tech%20Man_AD2_v100708.pdf
- 3 Zhang J et al.: (1999) *J. Biomol. Screen.* 4 (2), 67-73.

Transcreener® is a patented technology of BellBrook Labs.

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