

Transcreener® ADP Fluorescence Polarization Assay Performed on the PHERAstar

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Introduction

The use of high throughput screening for discovery of new kinase inhibitors is a fundamental approach being pursued for many diseases. Conventional kinase HTS assays rely on detection methods specific for a single phosphorylated product. This is problematic in that each of the many kinase subfamilies must then have its own unique set of assay reagents, specifically optimized.

To eliminate these difficulties, BellBrook Labs has developed the universal Transcreener® ADP Assay, a homogeneous, competitive fluorescent polarization HTS assay that directly detects ADP, the invariant reaction product of all kinase reactions. A single set of reagents, which includes a novel anti-ADP antibody and a far red ADP Alexa633 tracer, can be used to assay across the entire protein kinase family as well as the lipid kinase and carbohydrate kinase families. Rapid generation of meaningful and comparable data is obtained using standardized assay methodologies and data analyses. The Transcreener® ADP Assay is not dependent on a modified acceptor substrate, and therefore is not limited to use with kinases, but can be used to assay any ADP-producing enzyme, including ATPases, carboxylases, and helicases. Likewise, the assay can be easily optimized to accommodate a range of ATP concentrations¹.

BMG LABTECH's PHERAstar is a multifunctional microplate reader that combines rapid plate reading necessary for HTS with enhanced performance and sensitivity needed to read small fluid volumes. The PHERAstar reads all HTS detection modes (fluorescence intensity, timeresolved fluorescence, fluorescence polarization, luminescence, and absorbance) in all plate formats up to 1536 wells. The PHERAstar uses a unique application-specific module in conjunction with an optical reading head featuring five photo-

multiplier tubes that can simultaneously measure two emission signals at any desired wavelength. This optical design provides for outstanding sensitivity and accuracy in fluorescence and luminescence assays, and the simultaneous measurement minimizes the read time.

Assay Principle

The Transcreener® ADP Assay is a fluorescence polarization immuno-assay 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, kinases catalyze the transfer of phosphate from ATP to a protein, peptide, lipid or small molecule resulting in the accumulation of ADP.

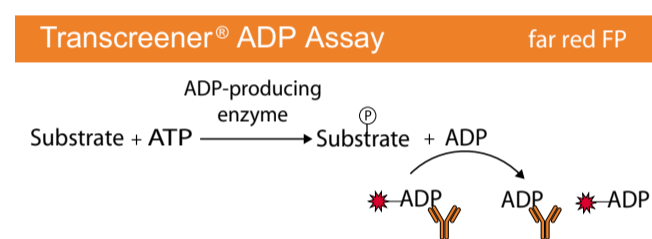


Fig. 1: Transcreener Assay Principle for Kinases

In step two the Transcreener® ADP 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

All materials were obtained through normal distribution channels from the manufacturers stated.

- Black 384 well and 1536 well microplates from Corning (#3676 and #3728)
- Transcreener® ADP Assay from BellBrook Labs, Madison, WI, Cat.No. 3004-1K (including ADP Far Red Tracer, ADP Antibody, Stop and Detect Buffer, ADP)
- Adenosine triphosphate (ATP) purchased from Sigma-Aldrich, Taufkirchen, Germany
- PHERAstar, BMG LABTECH, Offenburg, Germany
- ThermoStar, BMG LABTECH, Offenburg, Germany

Using 10 μM ADP and 10 μM ATP stock solutions a 15 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 μM ADP/10 μM ATP (mimicking

0% conversion) and the lower limit was set to 10 μM ADP/0 μM ATP (mimicking 100% conversion).

To the different ADP/ATP solutions the same volume of ADP Detection Mixture was pipetted. The antibody concentration for both well formats was 20 $\mu\text{g}/\text{mL}$. (For ideal assay performance it is important to determine an optimal antibody concentration under the specific enzyme and buffer conditions used in your experiment).¹ The concentration of the far-red tracer was 2 nM. The solutions (384 well final volume 20 μL , 1536 well final volume 5 μL) 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 figure 3 show the standard curves measured on the PHERAstar in 384 well and 1536 well format, respectively. Graphing on the log scale eliminates the point that corresponds to zero. To include all fifteen points along the curve, the value for 0 μM ADP/10 μM ATP was graphed at 0.01 μM position.

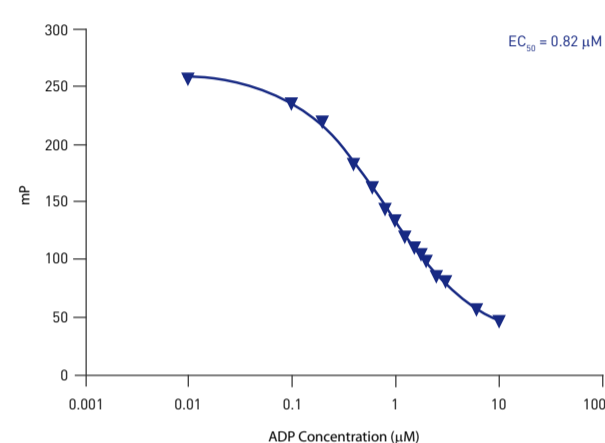


Fig. 2: ATP/ADP standard curve performed in a 384 well microplate.

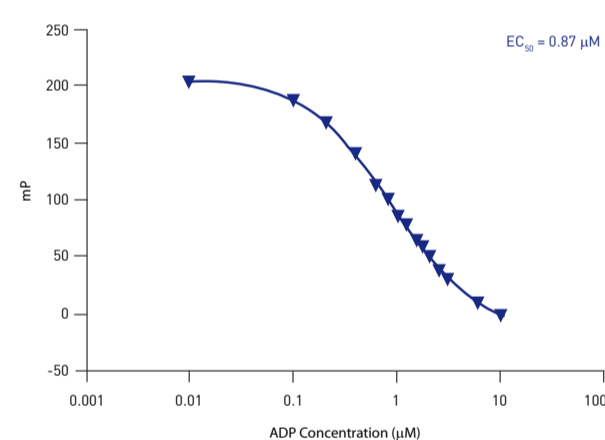


Fig. 3: ATP/ADP standard curve performed in a 1536 well microplate.

Both graphs show similar ranges and also similar EC_{50} values indicating that the Transcreener® ADP assay can be performed on the PHERAstar using both plate types. In order to show that there is no significant well to well variation, 24 replicates of the upper and lower limit of the standard curve were measured in a 384 well small volume plate.

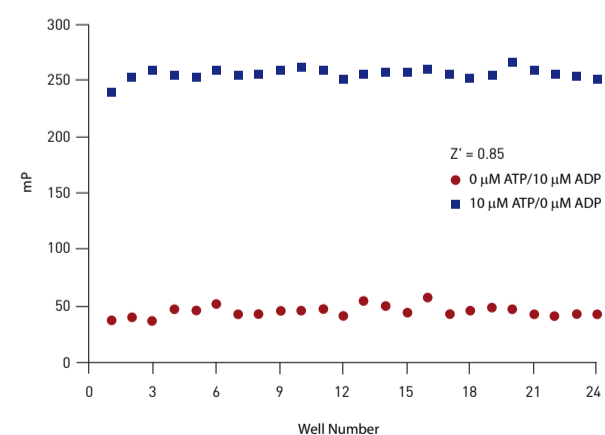


Fig. 4: Transcreener FP values for 24 replicates for the upper limit (●) and the lower limit (■) of standard curve

Figure 4 shows the high consistency of well to well measurements when using the PHERAstar for the Transcreener® ADP assay. In 384 well format, the standard deviation at the top and bottom limits are 5 mP, whereas in 1536 well format, the standard deviation at the top and bottom limits are 6 mP.

Conclusion

The universally generic nature of the Transcreener® ADP 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 of 0.85 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 instrumentation².

BMG LABTECH's PHERAstar microplate reader (Figure 5) provides the ideal platform for the Transcreener® ADP Assay. With its dual wavelength emission detection and five photomultiplier tubes (PMTs), the PHERAstar 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.

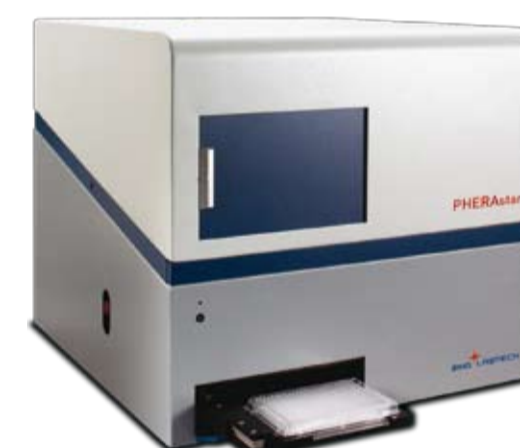


Fig 5. BMG LABTECH's PHERAstar - Multifunctional HTS microplate reader

References

- 1 Transcreener® ADP Assay Technical Manual, BellBrook Labs, Madison www.bellbrooklabs.com/PDFs/Tech%20Man_Kinase%20Plus_v091907.pdf
- 2 Zhang J et al.: (1999) *J. Biomol. Screen.* 4(2), 67-73.

Transcreener® is a patented technology of BellBrook Labs.

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