Transcreener™ KINASE Fluorescence Polarization Assay Performed on the PHERAstar

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Introduction

Kinases are important second messengers in many signal transduction pathways and are activated upon stimulation of a cell, which usually occurs through a receptor (i.e. G-protein coupled receptor or tyrosine kinase receptor). Therefore, the activation of a kinase is used in many high-throughput screens (HTS) as a readout for receptor stimulation[1].

Upon activation, kinases transfer a phosphate group from ATP to a substrate, thereby creating the by-product ADP. There are many kinds of kinases in the human body and they all have varying substrates. Most high-throughput screens examine a specific kinase and its substrate. This specificity requires the construction of an HTS assay for every kinase and substrate that wants to be studied, which can be very time consuming and costly. The Transcreener™ KINASE assay from BellBrook Labs avoids this problem by detecting the invariant by-product ADP[2].

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, time-resolved fluorescence, fluorescence polarization, luminescence, Alpha-screen™, 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 photomultiplier tubes that can simultaneous 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™ KINASE assay is a fluorescence polarization immunoassay based on the detection of ADP by an antibody (Figure 1).
This assay platform provides the possibility to detect enzymes that catalyze group transfer reactions with ADP.

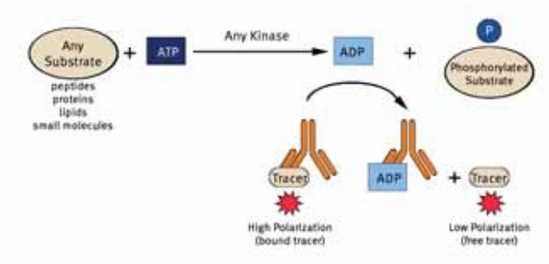

Fig. 1: Transcreener Assay Principle for kinases

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.

In step two the Transcreener™ ADP Detection Mixture, which contains an ADP Alexa Fluor 633 tracer bound to an anti-ADP antibody, is added. If there is enzymatic activity resulting in free ADP, 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 is proportional to a decrease in fluorescence polarization.

Materials and Methods

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

- 96 well and 384 well small volume, black microplates from Greiner Bio-One, Frickenhausen, Germany
- Transcreener™ Kinase Assay from BellBrook Labs, Madison, WI, Cat.No. 3003-1K (including ADP Far Red Tracer, ADP Antibody, Stop and Detect Buffer, ADP)
- Adenosintriphosphate (ATP) purchased from Sigma-Aldrich, Taufkirchen, Germany
- PHERAstar, BMG LABTECH, Offenburg, Germany (Figure 5)
- Thermostar, BMG LABTECH, Offenburg, Germany

Using 100 µM ADP and 100 µM ATP stock solutions a 15 point ADP/ATP standard curve was prepared, while keeping a constant concentration of total adenosine.

This standard curves mimics a kinase or ATPase reaction (ADP is produced while ATP is depleted).

The upper limit of the standard curve was set to 0 µM ADP/100 µM ATP (mimicking 0% conversion) and the lower limit was set to 100 µM ADP/0 µM ATP (mimicking 100 % conversion).

To the different ADP/ATP solutions the same volume of ADP Detection Mixture was pipetted into the microplate containing the suitable amount of antibody (for more details please refer to the assay protocol[2]). The solutions were mixed and incubated for 1 hour at room temperature. 300 µl was the final volume in a 96-well microplate and 20 µl was the final volume in a 384-well small volume plate.

The fluorescence polarization measurements were done using a 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 200 mP for the highest ATP concentration.

Results

Figure 2 and figure 3 show the standard curves measured on the PHERAstar in 96 well and 384 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/ 100 µM ATP was graphed at 0.02 µM position.

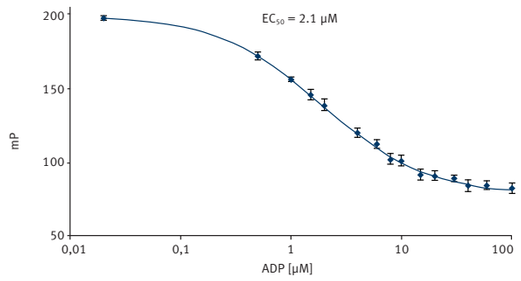

Fig. 2: ATP/ADP standard curve perfomed in a 96 well microplate.

Both graphs show similar ranges and also similar EC_{50} values indicating that the Transcreener™ Kinase FP assay can be performed on the PHERAstar using both plate types.

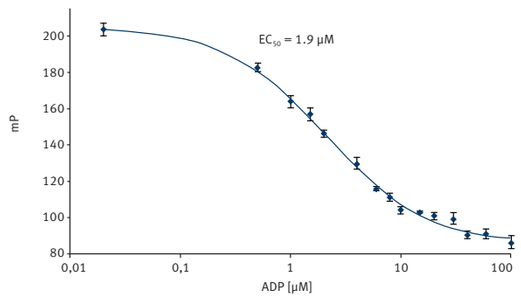

Fig. 3: ATP/ADP standard curve perfomed in a 384 well microplate.

In order to show that there is no significant well to well variation, 20 replicates of the upper and lower limit of the standard curve were measured in a 384-well small volume plate (Figure 4).

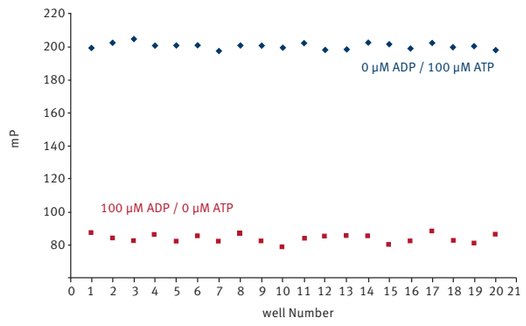

Fig. 4: Transcreener FP values for 20 replicates for the upper limit (◆) and the lower limit (■) of standard curve

Figure 4 shows the high consistency of well to well measurements when using the PHERAstar for the Transcreener FP assay. The resulting CVs (‹ 1 %CV for the upper limit and ‹ 3 %CV for the lower limit of standard curve) also demonstrate consistent measurements. From these data, a representative Z´value of 0.89 was calculated.

Conclusion

The generic nature of the Transcreener™ HTS Assay platform will eliminate delays involved in assay development for new HTS targets, and will greatly simplify compound and inhibitor profiling across multiple family members.

As a characteristic parameter for the quality of the assay, a Z'value of 0.89 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].

BMG LABTECH's PHERAstar microplate reader provides the ideal platform to simplify the Transcreener™ KINASE 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 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 Doerig C, Meijer L, Mottram JC. (2002) Protein kinases as drug targets in parasitic protozoa. Cell Signal. 15 (3), 243-253.

2 Transcreener™ KINASE Assay Technical Manual, BellBrook Labs, Madison WI. http://www.bellbrooklabs.com/pdf_files/BBL%20Protocol_KINASEplus_easyread.pdf.

3 Zhang J et al.: (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J.Biomol. Screen. 4, 67-73.

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