

New Transcreener® ADP² FP Assay performed on BMG LABTECH's POLARstar Omega

Franka Ganske, BMG LABTECH, Offenburg, Germany; E.J. Dell, BMG LABTECH, Durham, USA

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 μM).

The POLARstar Omega is a versatile, automated microplate reader offering seven detection modes (UV/Vis absorbance spectra, fluorescence intensity, fluorescence polarization, time-resolved fluorescence, time-resolved FRET, AlphaScreen® and flash and glow luminescence). For FP assays, the Simultaneous Dual Emission feature is very useful, providing the possibility to measure both the parallel and perpendicular channel simultaneously. That decreases the read times by two and corrects for flash to flash variations. In this poster we describe the use of the POLARstar Omega to perform the Transcreener® ADP² FP assay in a 96-well format.

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.

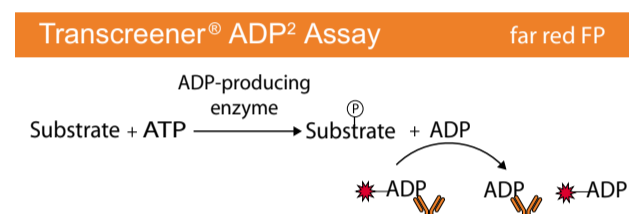


Fig. 1: Transcreener ADP² Assay Principle for Kinases

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 96 well half area flat bottom polystyrene NBS™ microplate, Corning (#3686)
- 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)
- Kemptide, AnaSpec (#22594)
- Protein kinase A (PKA), Promega (#V5161)
- POLARstar Omega, BMG LABTECH, Offenburg, Germany

Using 10 μM ADP and 10 μM 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 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 was 7.4 μg/mL. (For ideal 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 (96 well final volume 140 μL) were mixed and incubated for 1 hour at room temperature.

The fluorescence polarization measurements were performed using a 630-10 nm filter for excitation and a 670-10 nm filter for both emission channels. The mP target was set to 20 mP for the free tracer.

PKA enzyme titration

PKA was serially titrated 1:4 across the half area 96-well plate starting from 2000 mU/rxn leading to a final volume of 35 μL. 35 μL of 20 μM ATP was then added to the enzyme dilution. The plate was mixed for 30 seconds and then incubated for 60 minutes at room temperature. After that 70 μL of detection mix was then added to the wells. The plate was mixed as before and incubated once again for 60 minutes at room temperature.

Results and Discussion

Figure 2 shows the ADP/ATP standard curve measured on the POLARstar Omega in the 96 well format. To include all twelve points along the curve, the value for 0 μM ADP/10 μM ATP was graphed at 0.01 μM position.

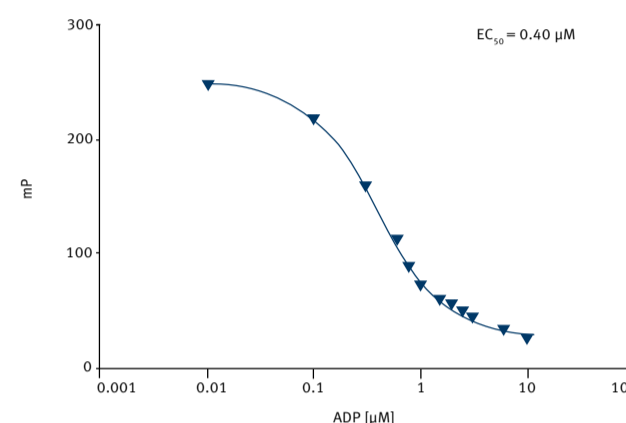


Fig. 2: ATP/ADP standard curve performed in a 96 well half area microplate

The calculated EC₅₀ value corresponds with data obtained with the PHERAstar Plus HTS microplate reader.³

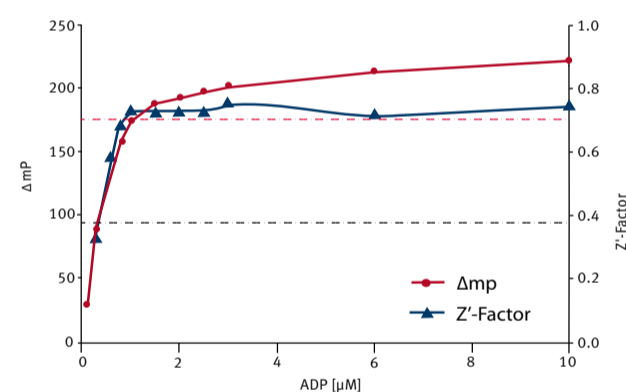


Fig. 3: Z' and ΔmP depending on the conversion.

The Z' validation minimal qualification is shown by the red dashed line (Figure 3). ΔmP validation qualification is shown by the black dashed line. A Z' > 0.7 and a mP shift of 173 units is achieved at 1.0 μM ADP (10 % ATP conversion).

PKA titration

The Transcreener® technology was applied to perform a PKA assay using Kemptide as substrate (Fig. 5). Kemptide is a phosphate acceptor peptide that serves as a synthetic substrate for PKA. The enzyme phosphorylates Kemptide by using a phosphate group from ATP that is converted to ADP.

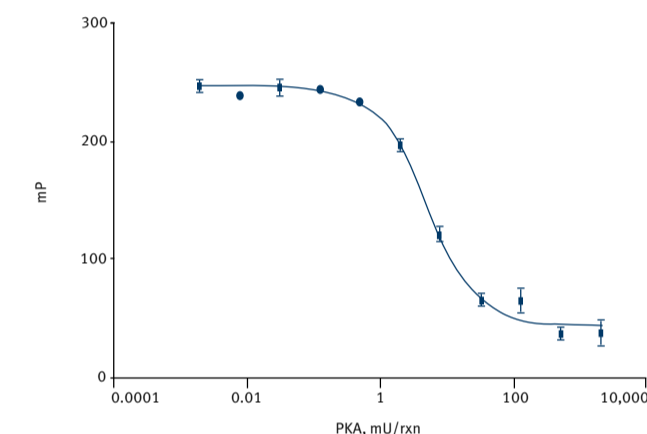


Fig. 4: PKA enzyme titration using a black 96 well half area plate.

A unit is defined as the amount of enzyme that is required to incorporate 1 pmol of phosphate into substrate in one minute. Figure 5 shows a PKA titration curve. A big assay window and small error bars indicate that the POLARstar Omega is well suited to perform any Transcreener® FP assay.

Conclusion

The data show that the POLARstar Omega passes the validation criteria for the Transcreener® FP assay in 96 well format.

With the ability to collect fast, full spectrum absorbance scans, to monitor endpoint, slow, and fast kinetics, the POLARstar Omega fulfills all assay needs. Top and bottom plate reading, multi-color detection, well scanning, precise temperature control, multimode shaking capabilities, a gas vent and onboard injectors enhance the flexibility for any application.



Fig. 5: BMG LABTECH's POLARstar Omega microplate reader

References

- BMG LABTECH Application note 152: Transcreener® ADP Fluorescence Polarization Assay Performed on the PHERAstar
- Transcreener® ADP² FP Assay Technical Manual, BellBrookLabs. Madison http://www.bellbrooklabs.com/PDFs/Tech%20Man_AD2_v100708.pdf
- BMG LABTECH Application note 182: New Transcreener® ADP² FP Assay performed on BMG LABTECH's PHERAstar Plus HTS Microplate Reader

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Germany: BMG LABTECH GmbH Tel: +49 781 96968-0

Australia: BMG LABTECH Pty. Ltd. Tel: +61 3 59734744
France: BMG LABTECH SARL Tel: +33 1 48862020
Japan: BMG LABTECH JAPAN Ltd. Tel: +81 48 647 7217
UK: BMG LABTECH Ltd. Tel: +44 1296 336650
USA: BMG LABTECH Inc. Tel: +1 919 806 1735

Internet: www.bmglabtech.com info@bmglabtech.com