The CLARIOstar® surpasses the certification needed for the Transcreener® ADP² FP assay

Franka Maurer, BMG LABTECH, Ortenberg, Germany
Meera Kumar, Bellbrook Labs, Madison, WI, USA

BMG LABTECH
The Microplate Reader Company

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- Transcreener® ADP² FP Assay can monitor any enzyme activity related to ADP production
- The CLARIOstar® was utilized to scan the tracer labeled antibody with the help of the new LVF monochromator
- Optimized filters were used to perform the FP assay with great sensitivity and high Z´values

Introduction

BellBrook Labs offers a variety of high throughput screenings assays for enzymes. The Transcreener® ADP² FP assay can be used to detect the activity of any kinase or ATPase. This simple, ADP detecting method is universal for all ADP-producing enzymes and can be used with any substrate.

In this application note we show ADP/ATP standard curves created with the Transcreener assay using the CLARIOstar® microplate reader from BMG LABTECH.

The CLARIOstar® is a new multimode microplate reader from BMG LABTECH that uses LVF monochromators, highly sensitive filters, and an ultra-fast spectrometer. The advanced monochromators in the CLARIOstar® have continually adjustable wavelengths and bandwidths (up to 100 nm) for excitation and emission.

Assay Principle

The Transcreener® FP Assays are a single step, competitive immunoassay for direct detection of nucleotides with a far red fluorescence polarization (FP) readout. The reagents for all of the assays are a far red tracer bound to a highly-specific monoclonal/ polyclonal antibody. Nucleotide diphosphate or monophosphate produced by the target enzyme displaces the tracer from the antibody, leading to increased rotational freedom and results in a decrease in polarization (Figure 1).

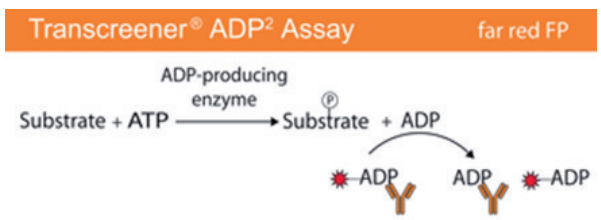

Fig. 1: Transcreener® ADP² Assay Principle for Kinases

The use of a far red tracer minimizes interference from fluorescent compounds and light scattering. The Transcreener® FP Assays are designed specifically for HTS with a single addition, mix-and-read format.

Materials and Methods

- ☐ Black 384-well small volume, non binding microplates from Greiner
- ☐ Transcreener® ADP² FP assay from Bellbrook Labs
- ☐ CLARIOstar® microplate reader (Fig. 2)

Fig. 2: The CLARIOstar® microplate reader from BMG LABTECH.

Filter selection

Fluorescence polarization measurements can be optimally performed using filters. To determine the ideal filter wavelengths, the Alexa633 tracer was spectrally scanned using the monochromator function of the CLARIOstar®. Although the fluorescence spectrum of Alexa633 is well known, assay specific conditions may have an influence on the spectral data. The resulting excitation and emission spectra are shown in figure 3.

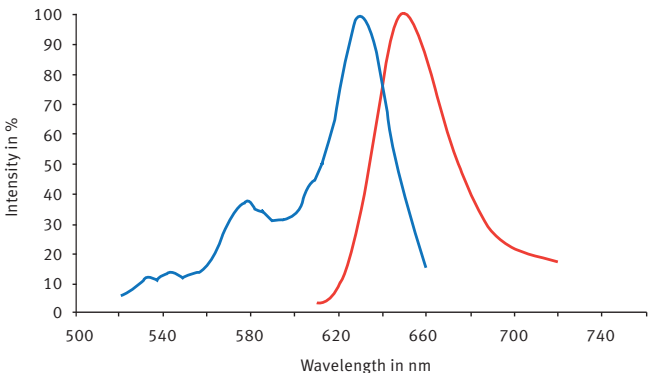

Fig. 3: ADP Alexa633 tracer from BellBrook Labs Transcreener® ADP² assay was scanned on the CLARIOstar® using LVF Monochromator. The excitation of tracer was scanned between 520 and 660 nm, a constant emission wavelength of 690 nm was used. The emission was scanned between 610 and 720 nm at a fixed excitation wavelength of 580 nm.

Based on the spectral data, the CLARIOstar® was equipped with a 590-50 filter for excitation and a 675-50 filter for emission in order to best perform the fluorescence polarization measurements.

Standards preparation

Transcreener® HTS assay performance were identified by running a 10 µM ATP/ADP and 0.1 µM ATP/ADP standard curve (24 replicates), as standard curves of this type mimic enzyme reactions. Starting with 10 µM or 100 nM ATP, ADP was added in increasing amounts and ATP is decreased proportionately, maintaining a total adenine nucleotide concentration of 10 µM and 100 nM respectively.

ADP Detection Mixture

This solution contains 4 nM tracer, 1x stop and detect buffer, and 15 µg/ml (for 10 µM) and 1 µg/ml (for 100 nM). The ADP detection mixture is diluted two fold in the well which leads to the following final concentrations in the well: 2 nM tracer, 0.5x buffer and antibody.

Antibody concentration

The final antibody concentration per well was 0.5 µg/ml (100 nM standard curve) and 7.5 µg/ml (10 µM standard curve). Please note that the optimal antibody concentration can differ significantly depending on the enzymatic reaction conditions. For optimal assay performance it is necessary to do an antibody titration under the specific enzyme and buffer conditions used in your experiment.

Instrument settings

10 µl of standard and 10 µl of detection mixture were mixed in the microplate which was sealed and incubated at room temperature for 1 hour. After incubation the sealer was removed and the plate was measured in the CLARIOstar® using the following instrument parameters:

Measurement Method:	Fluorescence Polarization, Endpoint Mode, Top optic
Filter Settings:	590-50 / LP 640 / 675-50
Settling time:	0.3 sec
Number of flashes:	50-200
Focus and gain:	adjusted prior the measurement
Target mP:	was set to be 20 mP for the free tracer (ADP Alexa633 tracer)

Results and Discussion

The two different sets of standards resulted in 4-parameter-fit standard curves. The low ADP concentration (100 nM) standard curve is shown in figure 4.

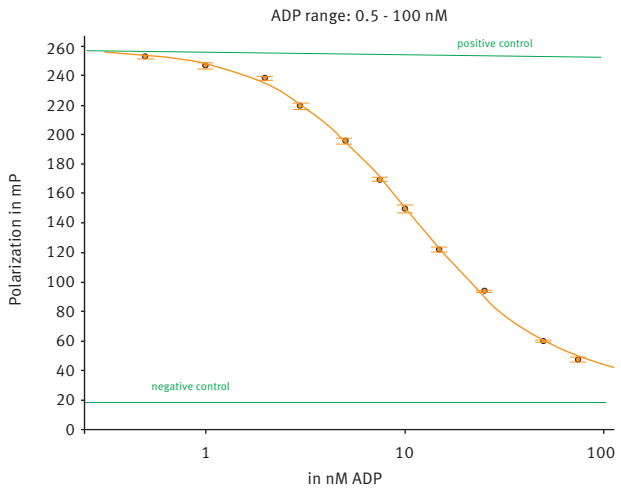

Fig. 4: 100 nM ADP/ATP standard curve measured on the CLARIOstar®

The standard curve fit results in an R^2 value of 0.9994. However, the curve does not reach the bottom level of the low polarization control. This can be avoided by using the optimal antibody concentration so further optimization would be useful. Because of this the assay window was calculated from the high FP control and the standard corresponding to 75 nM ADP leading to an assay window of 210 mP.

For Z' determinations the 10 µM ADP/ATP standard assay was performed by using › 10 replicates for each standard. This allowed us to show the Z' values over the concentration range.

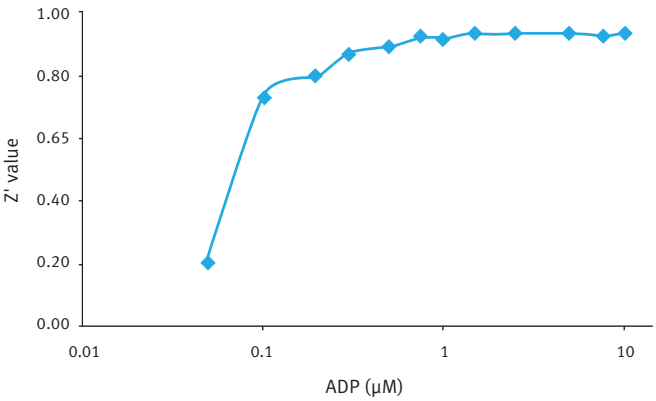

Fig. 5: Z' values over different ADP concentration for the 10 µM ADP/ATP standard curve.

At 10 % conversion (1 µM ADP) a Z' value › 0.9 is obtained. This indicates a very robust assay and proves the CLARIOstar® to be a reliable instrument for this assay.

Conclusion

Based on the data shown in this application note the CLARIOstar® was certified for the Transcreener® ADP² FP assay. This modular microplate reader is able to measure 8 detection modes, including: Fluorescence Intensity, FRET, Fluorescence Polarization, Luminescence, BRET, UV/Vis Absorbance, Time-Resolved Fluorescence, TR-FRET, and AlphaScreen/AlphaLISA® with a high energy laser. Reagent injectors and an integrated fluorophore library make the reader useful for assay development.

Transcreener® is a patented technology of BellBrook Labs.

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 48 86 20 20
Japan:	BMG LABTECH JAPAN Ltd.	Tel: +81 48 647 7217
UK:	BMG LABTECH Ltd.	Tel: +44 1296 336650
USA:	BMG LABTECH Inc.	Tel: +1 877 264 5227
Internet:	**www.bmglabtech.com**	**applications@bmglabtech.com**