Transcreener® ADP2 FP assay certification for BMG LABTECH instrumentation

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- Transcreener® ADP2 Assay kit is a far-red competitive FP immunoassay based on the detection of ADP
- Transcreener® can monitor any enzymatic reaction that produces ADP (ATP range is 0.1 - 1000 μM)
- PHERAstar® FS and CLARIOstar® validated by BellBrook Labs

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 during performing the Transcreener® assay using different microplate readers from BMG LABTECH.

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.

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 & Methods

- Black 384 well and 1536 well microplates from Corning
- Black 96 well half area flat bottom polystyrene NBS™ microplate, Corning

- Transcreener® ADP² Assay from BellBrook Labs, Madison, WI, (including ADP Alexa633 Tracer, ADP² Antibody, Stop & Detect Buffer B, ATP, and ADP)

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 a 384-well microplate which was sealed and incubated at room temperature for 1 hour. After incubation the sealer was removed and the plate was measured. For the 96-well half area plate the final volume in the well was 140 μl. For the 1536-well plate the final volume in the well was 8 μl.

<table>
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<th>Well Format</th>
<th>Detection mode</th>
<th>Method</th>
<th>Optic settings</th>
<th>mP target value</th>
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<td>Fluorescence Polarization</td>
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<td>Ex-Filter: 590-50 Em-Filter: 675-50</td>
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<td>1536-well</td>
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<td>94-well, 384-well, 1536-well</td>
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<td>NBS™ microplate, Corning</td>
<td>94-well</td>
<td>384-well, 1536-well</td>
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Fig. 1: Transcreener ADP Assay Principle for Kinases.

CLARIOstar® PHERAstar® FS

<table>
<thead>
<tr>
<th>Detection mode</th>
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<th>Optic settings</th>
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<tr>
<td>Fluorescence Polarization</td>
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<td>Transcreener specific FP optic module: FP 590 675 675</td>
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Keywords: ADP, Alexa633, ATP, competitive immunoassay, Transcreener
Results & Discussion

Figure 2 shows the ADP/ATP standard curve measured in the 96 well format. Graphing on the log scale eliminates the point that corresponds to zero. 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. Data was measured on a BMG LABTECH microplate reader equipped for fluorescence polarization measurement.

Figure 3 and 4 show the standard curves measured in 384 well and 1536 well format, respectively.

Fig. 3: ATP/ADP standard curve in a 384 well microplate. Data was measured on a PHERAstar FS.

Fig. 4: ATP/ADP standard curve in a 1536 well microplate. Data was measured on a PHERAstar FS.

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.

Based on the data exemplarily shown in this application note, the CLARIOstar as well as the PHERAstar FS were certified for the Transcreener® ADP² FP assay.

Transcreener® is a patented technology of BellBrook Labs.