The Transcreener® ADP² Universal Kinase Assay from BellBrook Labs is readily performed on BMG LABTECH microplate readers using different assay formats

Meera Kumar, BellBrook Labs, Madison, USA
EJ Dell, BMG LABTECH, NC, USA; Franka Ganske, BMG LABTECH, Offenburg, Germany

Introduction

The Transcreener® technology was developed by BellBrook Labs to quantify the production of ADP during enzyme reactions. Different detection modes are possible in combination with the Transcreener® method (FI, FP and TR-FRET). This poster focuses on the homogeneous, competitive red ADP² fluorescent intensity (FI) and fluorescence polarization (FP) assays. Herein we will show that combination of the Transcreener® chemistry with the PHERAstar *Plus* or PHERAstar *FS* as well as with the POLARstar or FLUOstar Omega microplate readers from BMG LABTECH provide excellent Z'values, indicating a robust assay and instrumentation.

Transcreener® ADP² FI Assay

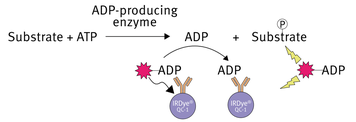

Fig. 1: Transcreener® ADP2 FI Assay Principle

After the enzymatic reaction, an ADP Alexa594 tracer bound to the ADP² monoclonal antibody, which is conjugated to an IRDye® QC-1 quencher (licensed from LI-COR®), is added. Accumulated ADP from the reaction will eventually displace the ADP-tracer from the antibody-quencher complex into the solution. Here the ADP-tracer complex becomes un-quenched resulting in an increase in fluorescence intensity. The ADP created during the enzyme reaction is proportional to the fluorescence signal.

Transcreener® ADP² Assay far red FP

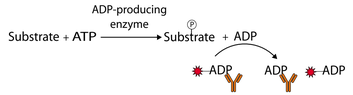

Fig. 2: Transcreener® ADP² FP Assay Principle

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 and Methods

Transcreener® ADP² FI Assay

To show the potential of the instrumentation, ADP/ATP standard curves were created to mimic an enzyme reaction. For that 10 µM ADP and 10 µM ATP stock solutions were combined to give 15 standards with an ADP range from 0 to 10 µM. For 96-well plates the reaction mix consisted of 25 µL of ADP/ATP dilution and 25 µL of ADP detection mixture. For 384-well plates 10 µL of each solution were combined. The final concentration of tracer in the well was 4 nM. The final concentration of antibody conjugated to the QC-1 quencher depends on the ATP concentration. For the 10 µM ADP/ATP dilutions a final antibody concentration of 5 µg/mL per well was used as recommended in the Transcreener® manual.[1]

As controls, a high RFU control and a low RFU control were prepared:
High RFU control = Positive control
4 nM tracer in 0.5x buffer

Low RFU control = Negative control
Detection mix, 4 nM tracer and 5 µg/mL antibody conjugated to the QC-1 quencher

After the addition of the detection mixture to the standards a one hour incubation at room temperature follows. The plate was then inserted into a plate reader, the gain was adjusted to 10% of the positive control and fluorescence was measured at 580/620 nm for excitation/emission.

Transcreener® ADP² Assay far red FP

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 assay 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 (PHERAstar readers: 384 well final volume 20 µL, 1536 well final volume 8 µL and POLARstar Omega: 96 well final volume 140 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 for the PHERAstar readers. The fluorescence polarization measurements in the POLARStar Omega were performed using a 630-10 nm filter for excitation and a 670-10 nm filter for both emission channels.

Results and Discussion

Transcreener® ADP² FI Assay

Figure 3 and 4 show a 15 point ADP/ATP standard curve monitored either on a PHERAstar or Omega plate reader in 384-well format.

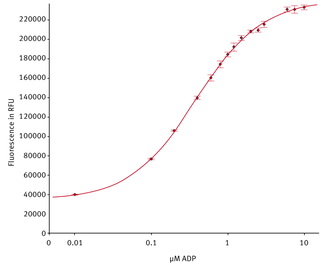

Fig. 3: 10 µM ADP standard curve measured in 5 replicates using a PHERAstar *Plus* in 384 well format (20 µL) utilizing the ADP² FI Assay.

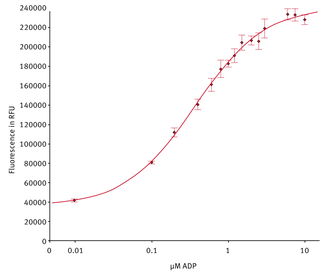

Fig. 4: 10 µM ADP standard curve measured in 5 replicates using a POLARstar Omega in 384 well format (20 µL) utilizing the ADP² FI Assay.

The standard curves for both instruments look very similar. Only the error bars are a bit higher in the Omega graph. This is due to the different optical systems used in PHERAstar and Omega instruments.

With the MARS Data Analysis Software that comes with every PHERAstar and Omega reader it is possible to calculate EC_{50} values from the 4-parameter fit of the standard curves. Table 1 shows these results and also the Z' values for both instrument series in comparison.

	PHERAstar *Plus/FS*	POLARstar/FLUOstar Omega
EC_{50} (µM) (96-well)	0.35	0.36
EC_{50} (µM) (384-well)	0.38	0.37
Z' value (384-well) at 10% conversion using 5 flashes	0.89	0.80

Transcreener® ADP² Assay far red FP

A representative standard curve for the PHERAstar *Plus* in 1536 well format is shown in Fig. 5. 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.

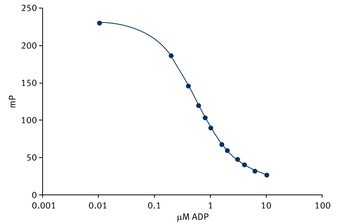

Fig. 5: ATP/ADP standard curve performed in a 1536 well microplate in a PHERAstar *Plus* utilizing the ADP² FP Assay.

Figures 6 and 7 show Z'and Δ mP values depending on the conversion for the PHERAstar *Plus* and POLARstar Omega, respectively.

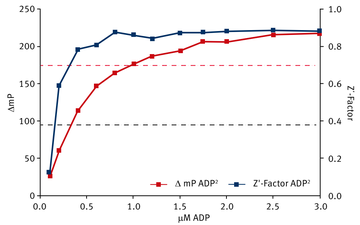

Fig. 6: Z' and Δ mP depending on the conversion for the PHERAstar Plus. Z' validation minimal qualification is shown by the red dashed line. Δ mP validation minimal qualification is shown by the black dashed line.

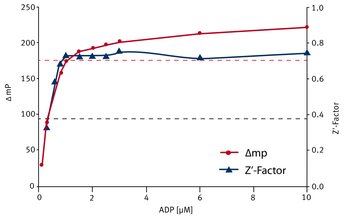

Fig. 7: Z' and Δ mP depending on the conversion for the POLARstar Omega. Z' validation minimal qualification is shown by the red dashed line. Δ mP validation minimal qualification is shown by the black dashed line.

The results show that the Transcreener® ADP² FP assay is able to yield higher quality data at conversion levels that lie within initial rate enzyme reaction kinetics.

Fig. 8: BMG LABTECH's multidetection microplate readers FLUOstar and POLARstar Omega

Fig. 9: BMG LABTECH's multidetection microplate readers PHERAstar *FS* and *Plus*

Conclusion

The universally generic nature of the Transcreener® ADP² assay will reduce assay development efforts thus allowing HTS to occur earlier. As a characteristic parameter for the quality of the assay, Z'values were calculated, which represents an excellent assay performance for both the ADP² FI and the ADP² FP assay.

We show that the Transcreener® ADP² FI assay is compatible with four different microplate readers from BMG LABTECH. The PHERAstar *FS* and *Plus*, as well as the POLARstar and FLUOstar Omegas show similar standard curves and EC_{50} values. A Z' of 0.89 and 0.80 were calculated for the PHERAstar and Omega instruments, respectively, indicating a good quality assay and robust instrumentation.

The POLARstar Omega passes the validation criteria for the Transcreener® FP assay in 96 well format as well as the PHERAstar readers in 384-well format. The PHERAstar *Plus*/*FS* microplate reader provides the ideal platform for the Transcreener® ADP² Assay. With its dual wavelength emission detection and five photomultiplier tubes (PMTs), the PHERAstar *Plus* 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.

References:

1. http://www.bellbrooklabs.com/PDFs/Tech%20Man_ADP²%20FI_v060809.pdf
2. Transcreener® ADP² FP Assay Technical Manual, BellBrookLabs. Madison http://www.bellbrooklabs.com/PDFs/Tech%20Man_ADP2_v100708.pdf

Don't miss our microplate readers!

Visit BMG LABTECH's booth 817 and see BellBrook Labs at booth 310.