The Transcreener® ADP² FI Assay performed on PHERAstar and Omega microplate readers

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- Transcreener® ADP² FI assay kit is a simple one-step competitive red fluorescence immunoassay based on the detection of ADP
- PHERAstar *Plus*, PHERAstar *FS*, POLARstar Omega and FLUOstar Omega microplate readers from BMG LABTECH are compatible with this assay

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 application note focuses on the homogeneous, competitive red ADP² fluorescent intensity (FI) assay.

The assay is based on the detection of ADP, therefore is compatible with any enzyme class that produces ADP, including protein, lipid, and carbohydrate kinases, ATPases, DNA helicases, carboxylases and glutamine synthetases. The assay is a simple one step homogeneous detection assay that can be applied to a wide range of ATP concentrations (0.1 to 100 µM ATP).

In this application note 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 provide excellent Z'values, indicating a robust assay and instrumentation.

Fig. 1: BMG LABTECH's multidetection microplate readers - PHERAstar *FS* and POLARstar Omega

Assay Principle

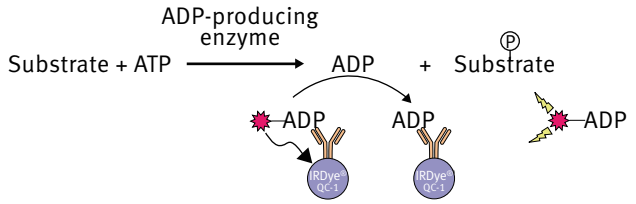

Fig. 2: Transcreener® ADP² FI Assay Principle

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.

Materials and Methods

- ☐ Black 96-well half area plates from Corning, UK
- ☐ Black 384-well small volume plates from Greiner bio-one, Germany
- ☐ Transcreener ADP² FI Assay Kit for 96-wells or 384-wells from BellBrook Labs
- ☐ Microplate reader from BMG LABTECH (PHERAstar *FS*, PHERAstar *Plus*, POLARstar Omega or FLUOstar Omega are compatible)

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:

<u>High RFU control = Positive control</u>
4 nM tracer in 0.5x buffer

<u>Low RFU control = Negative control</u>
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.

Data Analysis

The Z'-value, a standard for evaluating HTS methods, is calculated using the formula:

$$Z' = 1 - \frac{3\sigma_p + 3\sigma_n}{|\mu_p - \mu_n|}$$

where μ_p = mean of "positive control" (max ratio), μ_n = mean of "negative control" (min ratio), and σ = the corresponding standard deviations.

Results and Discussion

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

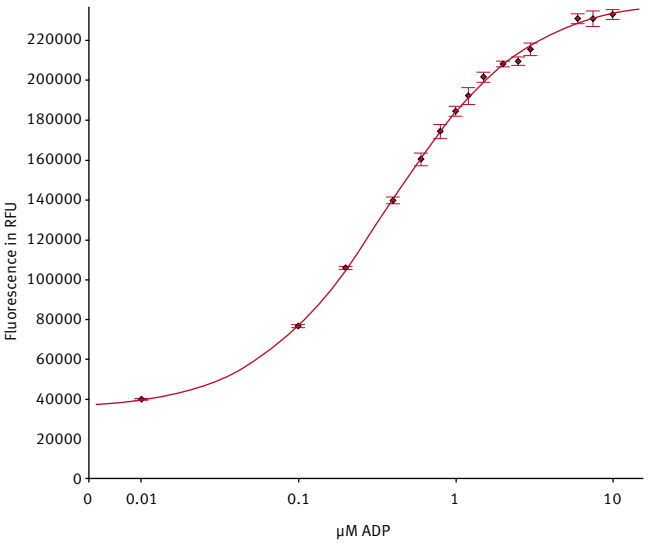

Fig. 3: 10 µM ADP standard curve measured in 5 replicates using a PHERAstar *Plus* in 384 well format (20 µL). The concentration of 0 µM ADP was set to 0.01 µM to allow logarithmic scaling.

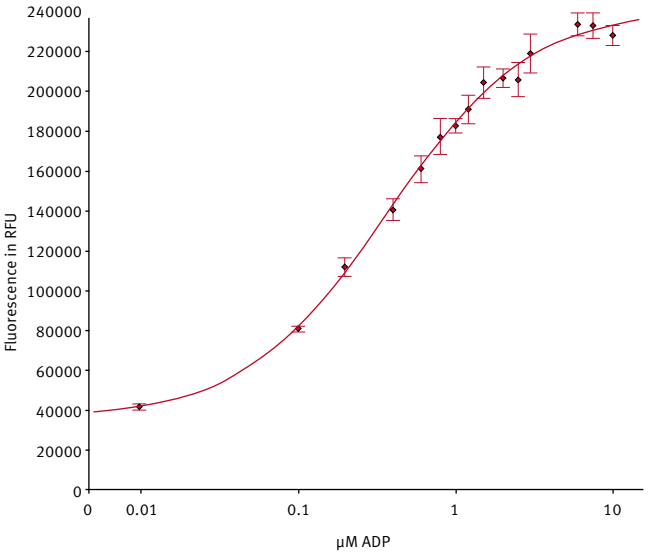

Fig. 4: 10 µM ADP standard curve measured in 5 replicates using a POLARstar Omega in 384 well format (20 µL). The concentration of 0 µM ADP was set to 0.01 µM to allow logarithmic scaling.

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. This effect can also be observed in figure 5. Here the Z'values shown are dependent on the number of flashes.

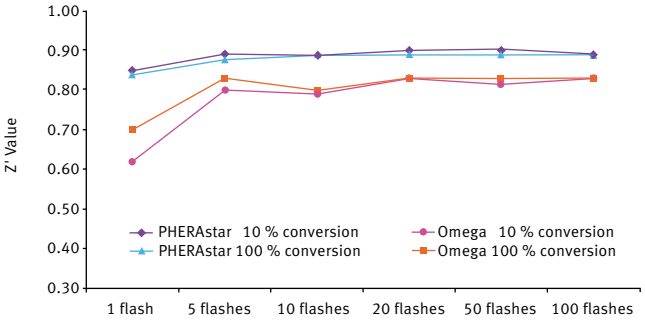

Fig. 5: Z'values obtained with the PHERAstar and Omega are dependent on the number of flashes. For the Z'calculation at least 20 replicates of ADP or ATP dilution were used.

Figure 5 shows that with the PHERAstar it is possible to use only 1 flash to get excellent Z'values (› 0.8). For the Omega instruments at least 5 flashes should be used.

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.

Table 1: EC_{50} values in 96-well and 384-well format and Z'values compared for PHERAstar and Omega microplate readers

	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

Comparable EC_{50} values were obtained with all instruments in 96-well and 384-well format. The Z'values are very good for the Omega plate readers and even slightly better for the PHERAstar instruments. This is in accordance to the HTS optical system that is used exclusively in the PHERAstar plate readers.

Conclusion

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.

References

1. http://www.bellbrooklabs.com/PDFs/Tech%20Man_ADP²%20FI_v060809.pdf

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