BMG LABTECH

Transcreener® ADP² FI assay performed on BMG LABTECH 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**
- **BMG LABTECH microplate readers 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 BMG LABTECH microplate readers provide excellent Z'values, indicating a robust assay and instrumentation.

Assay Principle

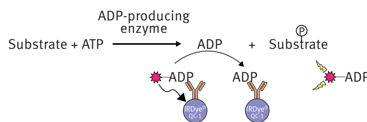

Fig. 1: Transcreener® ADP² FI Assay Principle.

After the enzymatic reaction is finished, 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 & Methods

- 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

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 384-well plates the reaction mix consisted of 10 µL of ADP/ATP dilution and 10 µL of ADP detection mixture. 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.

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.

Instrument settings

	POLARstar® Omega	CLARIOstar®	PHERAstar® *FS*
Detection mode	Fluorescence Intensity		
Method	Endpoint, Top optic		
Optic settings	Excitation Filter: 580-10 Emission Filter: 620-10	Monochromator settings 575-20/630-40	Transcreener specific FI optic module: FI 580 620

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.

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Results & Discussion

Figure 2 and 3 show a 15 point ADP/ATP standard curve monitored either on the PHERAstar FS or Omega plate reader in 384-well format.

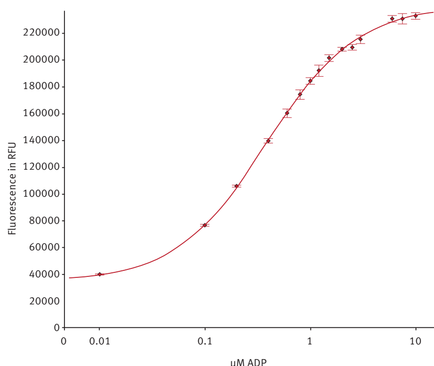

Fig. 2: 10 µM ADP standard curve measured in 5 replicates using a PHERAstar *FS* in 384 well format (20 µL). The concentration of 0 µM ADP was set to 0.01 µM to allow logarithmic scaling.

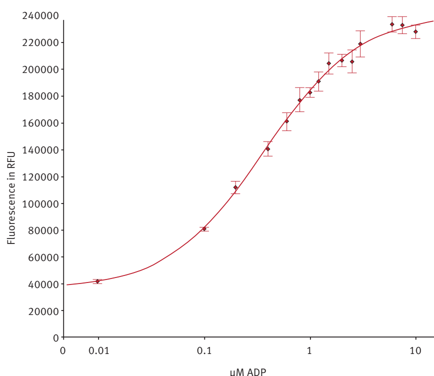

Fig. 3: 10 µM ADP standard curve measured in 5 replicates using a POLARstar Omega in 384 well format (20 µL). The concentration of 0 µM ADP was set to 0.01 µM to allow logarithmic scaling.

The standard curves for PHERAstar *FS* and CLARIOstar look very similar (data for CLARIOstar not shown). For the Omega the error bars are a slightly higher in the standard curve. This is due to the different optical system used in Omega instruments.

With the MARS Data Analysis Software that comes with every BMG LABTECH reader it is possible to calculate different assay parameters, like Z´values with just one mouse-click. Table 1 summarizes Z´value results for the different instrumentation.

Table 1: Z´ values for the Transcreener assay compared for different BMG LABTECH instrumentation.

	PHERAstar *FS*	CLARIOstar	POLARstar/ FLUOstar Omega
Z´value (384-well) at 10 % conversion using 5 flashes	0.89	0.83	0.80

Monochromator-based instruments like the CLARIOstar are often thought to have a significant higher read time to perform assays compared to filter-based microplate readers. The read time is depending on the number of flashes. In Table 2 this relation is shown as well as the corresponding Z' values.

Table 2: CLARIOstar® assay performance at 10 % conversion 10 µM ATP.

Flashes	1	5	10	20	50
Read Times (min) whole 384 well plate	1:23	1:31	1:43	2:02	3:01
Z'Factor at 10 % ATP conversion	0.67	0.83	0.85	0.87	0.89

The criteria to get the Transcreener® FI certification is to achieve a Z' factor of 0.7 at 10 % ATP conversion. As shown in Table 2 this Z' value is already reached if only 5 flashes are used resulting in a read time of 1:31 for a whole 384-well plate. This indicates that measurements with the CLARIOstar using the LVF monochromators are fast and reliable at the same time.

Conclusion

We show that the Transcreener® ADP² FI assay is compatible with the PHERAstar FS, the CLARIOstar and the POLARstar and FLUOstar Omega.

PHERAstar® *FSX*

*The PHERAstar *FSX* is the newest PHERAstar reader.

CLARIOstar®

Omega Series