Fluorescence Intensity Transcreener® assay used to detect ADP on the CLARIOstar®

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The Microplate Reader Company
Application Note 245, Rev. 09/2013

- Any enzyme reaction that produces ADP can be followed using the Transcreener® ADP² FI assay
- Fluorescence excitation and emission scans of tracer were done on the CLARIOstar® to obtain optimal measurement settings
- High Z´values at 10 % conversion allowed the CLARIOstar® to become Transcreener® FI certified

Introduction

The Transcreener® ADP² fluorescent intensity (FI) Assay from BellBrook Labs results in a fluorescent intensity output that can be used to quantify enzyme activity. Any enzyme reaction that creates ADP as a byproduct can be monitored that way, allowing determining activities of e.g. protein, lipid, and carbohydrate kinases, ATPases, DNA helicases, carboxylases and glutamine synthetase.

The Transcreener ADP² Assay is a simple one-step homogenous detection assay and is flexible with regard to ATP concentration (0.1 to 100 µM ATP). The assay was performed on the CLARIOstar® multimode microplate reader from BMG LABTECH.

Assay Principle

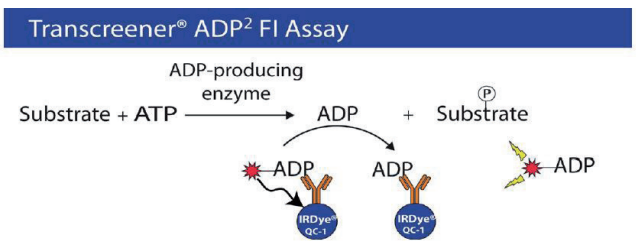

Fig. 1: Transcreener ADP² FI assay principle

The Transcreener ADP Detection Mixture comprises a quenched ADP Alexa594 Tracer bound to the ADP² monoclonal antibody conjugated to an IRDyeR QC-1 quencher licensed from LI-COR® (Fig. 1). The tracer is displaced by ADP, the invariant product generated during an enzyme reaction. The displaced tracer becomes un-quenched in solution leading to a positive increase in fluorescence intensity. Therefore, ADP production is proportional to an increase in fluorescence. The red tracer minimizes interference from fluorescent compounds and light scattering.

Materials and Methods

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

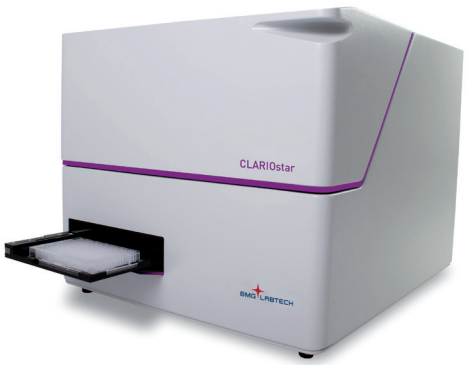

Fig. 2: The CLARIOstar® microplate reader from BMG LABTECH.

Monochromator settings

To obtain the optimal monochromator settings for the assay, a fluorescence spectrum was taken from the tracer that is coupled to the fluorescent dye Alexa594. The spectra for excitation and emission are shown in figure 3.

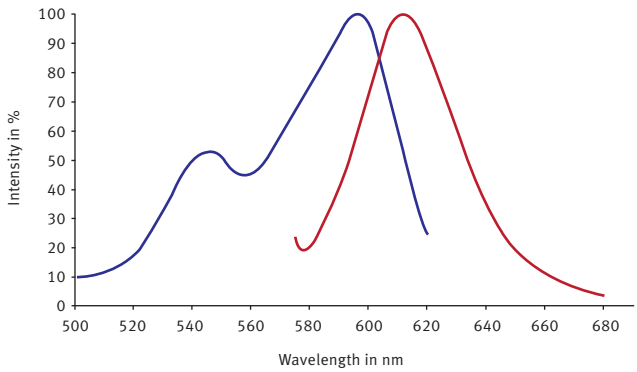

Fig. 3: Alexa594 tracer from BellBrook Labs Transcreener® ADP² FI assay was scanned on the CLARIOstar®. The excitation of tracer was scanned between 500 and 620 nm at a constant wavelength of 660 nm. The emission was scanned between 575 and 680 nm at a fixed excitation wavelength of 545 nm.

Based on the spectral data the following monochromator settings were chosen: excitation wavelength 577 nm with a bandwidth of 26 nm and emission wavelength 641 nm with a bandwidth of 44 nm.

ADP/ATP Standard preparation

ADP/ATP standards were prepared in order to show the ability of the instrument to detect a wide range of ADP. 10 µM ADP and 10 µM ATP stock solutions were combined to give 15 standards with increasing ADP concentrations ranging from 0 to 10 µM.

High and Low RFU control

These controls are intended to check if the instrument can work in an appropriate fluorescence window. The high RFU control contains tracer and buffer while the low RFU control contains tracer and antibody. The ratio of high to low RFU mixture should be › 5.

Plate preparation and instrument settings

10 µl of standard and 10 µl of ADP detection mixture (containing ADP2 antibody-IRDye QC-1 and ADP Alexa594 tracer) are combined in the microplate and incubated for 1 hour at room temperature. After incubation the plate was measured on the CLARIOstar® using the following settings:

Measurement Method:	Fluorescence Intensity, Endpoint Mode, Top optic
Monochromator settings	
-Excitation wavelength (nm):	575
-Excitation Bandwidth (nm):	20
-Emission wavelength (nm):	630
-Emission bandwidth (nm):	40
Settling time (s):	0.1
Flashes:	1, 5, 10, 20 and 50
Gain and Focus:	adjusted prior to the measurement

Results and Discussion

After the measurements a 4-parameter fit standard curve was prepared with background corrected values (Fig. 3).

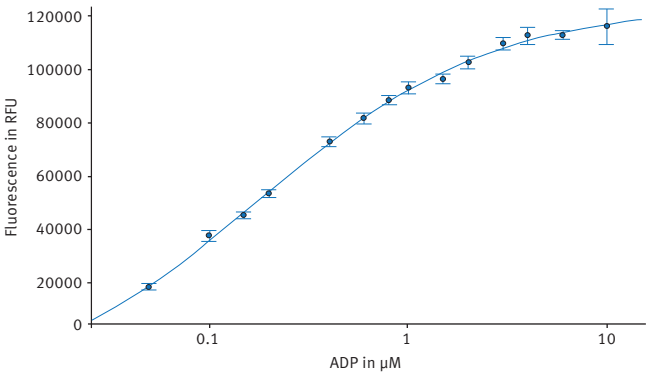

Fig. 4: 10 µM ATP/ADP standard curve, corrected for the 100 % ATP sample

Low error bars and an R2 value of 0.998 indicate a very good readout on the CLARIOstar.

Well consistency and instrument to instrument variation was determined by running the assay with 20 replicates on 4 different CLARIOstar® microplate readers. Fig. 4 shows the variation over replicates and instruments.

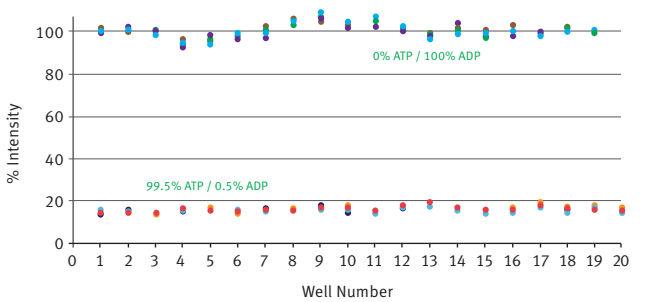

Fig. 5: Well to well variation for 20 replicates. Data is subtracted by background (100 % ATP) and normalized to the average intensity of the 100 % ADP sample.

The sample values for all 4 CLARIOstar® instruments are very close indicating that the performances of the readers are comparable. Variations between replicates are therefore mostly caused by manual pipetting and meniscus effects.

Monochromator-based instruments 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 1 this relation is shown as well as the corresponding Z' values.

Table 1: CLARIOstar® assay performance at 10 % conversion 10 µM ATP

Flashes	1	5	10	20	50
Read Times (min) whole 384w 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 1 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. The fastest of BMG LABTECH's filter-based instruments, the PHERAstar FS, is only 4 seconds faster using 5 flashes. This indicates that measurements with the CLARIOstar® using the LVF monochromators are fast and reliable at the same time.

Conclusion

The CLARIOstar® multimode microplate reader surpasses the certification needed for the Transcreener® ADP² FI Assay. The fluorescence intensity measurements using the LVF monochromators are carried out fast and reliably.

Transcreener® is a patented technology of BellBrook Labs.

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