Promega's ADP-Glo™ Kinase Assay performed on BMG LABTECH's PHERAstar *FS* and POLARstar Omega

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Application Note 202, Rev. 12/2009

- ADP-Glo™ Kinase Assay is a homogeneous luminescent assay to detect ADP
- ADP concentrations ranging from 1 mM to 0.01 µM can be detected
- Kit was successfully performed on the PHERAstar *FS* and POLARstar Omega using on-board injectors

Introduction

Kinases are a large and diverse group of enzymes that are involved in many cellular metabolic and regulatory processes. During the kinase reaction, substrates are phosphorylated while ATP is converted into ADP. Screening for active kinases or for kinase inhibitors is an important tool for the development of new drugs. To fulfil this need, Promega developed a kinase assay that is based on luminescence and the by-product ADP. The ADP-Glo™ Kinase assay is a universal, homogeneous, high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during the kinase reaction. This assay can be used to screen for any ADP-generating enzyme.

In this application note we will show results of the ADP-Glo Kinase assay obtained with the PHERAstar *FS* and POLARstar Omega multi-detection microplate readers from BMG LABTECH.

Assay Principle

The principle of the assay is presented in figure 1.

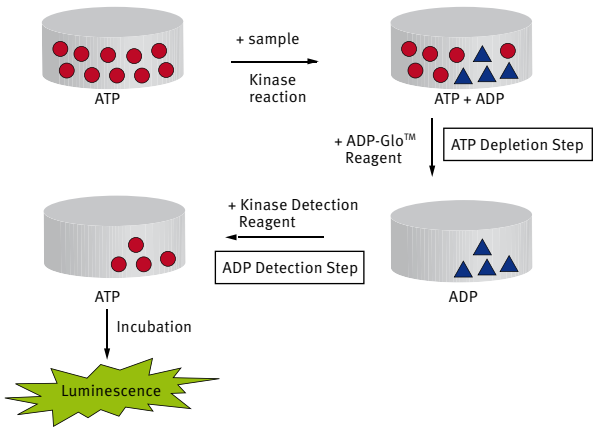

Fig. 1: Principle of the ADP-Glo™ Kinase Assay.

The assay consists of two steps. After the enzymatic reaction is finished, ATP and ADP are present in the well. The first step is to add the ADP-Glo™ Reagent resulting in the elimination of the remaining ATP. The second step is to convert the remaining ADP into ATP by adding Kinase Detection Reagent. This reagent also contains everything needed to measure the newly generated ATP with the help of a luciferase/luciferin reaction. The luminescence measured after incubation is proportional to the ADP concentration generated during the enzymatic reaction.

Materials and Methods

- ☐ White 384-well small volume plates from Greiner
- ☐ ADP-Glo™ Kinase Assay from Promega, including ATP and ADP
- ☐ PHERAstar *FS* or POLARstar Omega microplate reader (Fig. 2)

Fig. 2: BMG LABTECH's multidetection microplate readers PHERAstar *FS* and POLARstar Omega

To mimic an enzymatic reaction, an ADP/ATP standard curve was prepared from the nucleotide stock solutions that were supplied with the kit. The assay allows ADP measurements from 1 mM ADP to 0.01 µM ADP. Four different ADP/ATP standard curves were prepared: 1 mM, 100 µM, 10 µM and 1 µM. As an example, the dilution table for a 1 mM ATP/ADP standard dilution is given below. All other standard curves were diluted accordingly.

mM	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
ADP	1	0.8	0.6	0.4	0.2	0.1	0.05	0.04	0.03	0.02	0.01	0
ATP	0	0.2	0.4	0.6	0.8	0.9	0.95	0.96	0.97	0.98	0.99	1

Five µL of standard dilution was mixed with 5 µL of ADP-Glo™ reagent followed by a 40 min incubation at room temperature. After that 10 µL of Kinase Detection Reagent was then injected with onboard injectors. The signal was measured each minute for 40 minutes. Alternatively it is possible to measure endpoint luminescence after a 40-60 min incubation at room temperature.

Instrument settings for a kinetic reaction on a PHERAstar FS or POLARstar Omega

Detection Mode:	Luminescence, plate mode
Measurement time:	1 sec
Cycle Time:	60 sec
Cycles:	40
Optics:	Luminescence Module (PHERAstar *FS*)
	2 mm optic (POLARstar Omega)
Gain:	Optimized for the ADP concentration

Results and Discussion

Fig. 3 shows a signal curve taken from the PHERAstar *FS* for a 1 mM ADP/ATP standard dilution.

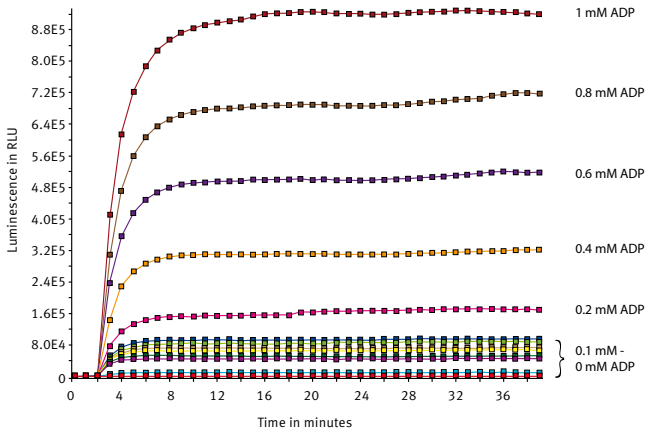

Fig. 3: Signal curves for several dilutions of ADP/ATP standard measured on the PHERAstar *FS*. The gain was set to 2400.

The signal curves show that already after 20 min the signal is very stable. The 12 standards show different signal heights according to their ADP concentration. The last 10 data points of the signal curves were averaged and taken as a base for a linear regression fit.

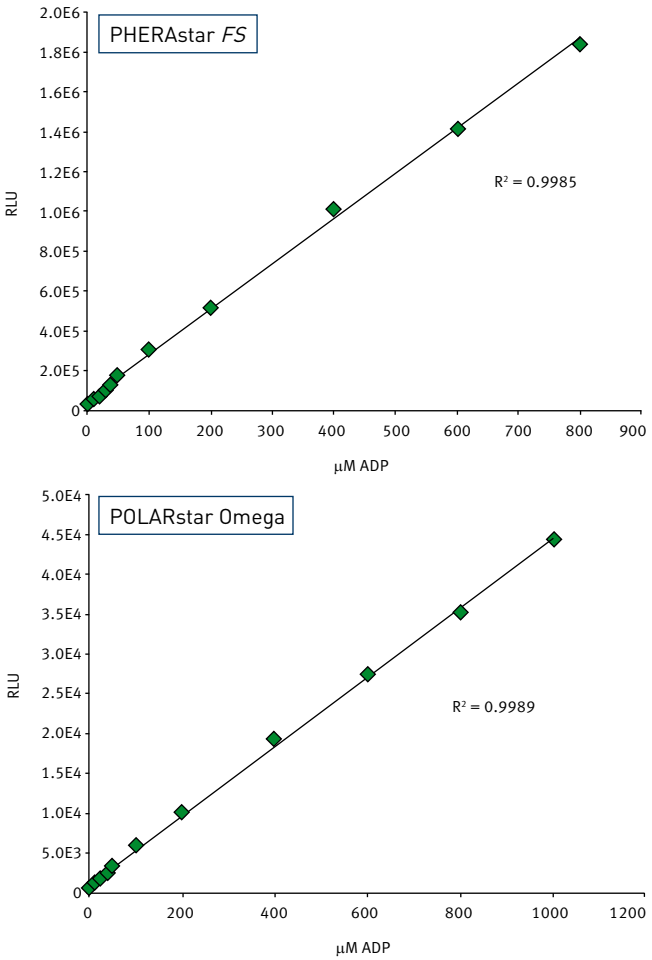

Fig. 4 and 5: One mM ADP/ATP standard curve for PHERAstar *FS* and POLARstar Omega.

These fits can be seen in figures 4 and 5 for a 1 mM nucleotide standard curve as well as in figures 6 and 7 for a 1 µM nucleotide standard curve.

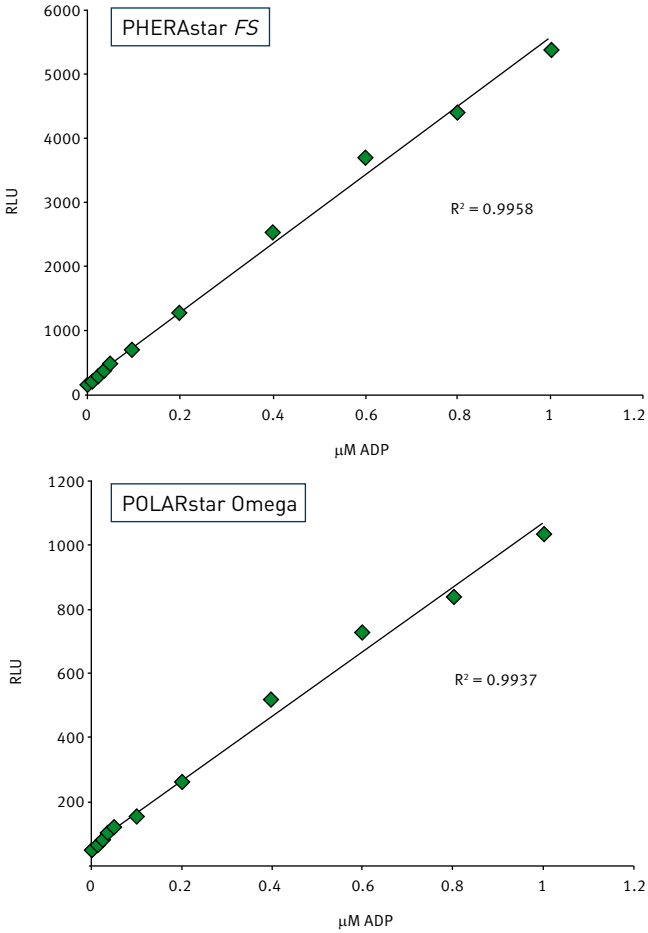

Fig. 6 and 7: One µM ADP/ATP standard curve for PHERAstar *FS* and POLARstar Omega.

The standards curves obtained on the PHERAstar *FS* and POLARstar Omega look similar, but note how the *FS* has a steeper slope at lower ADP concentration. This reflects the fact that the PHERAstar *FS* HTS instrument is more sensitive and has a better LOD, which is expected. It is possible to measure standard curves from 1 mM to 1 µM ADP using the same gain settings indicating a high assay window for both instruments.

Conclusion

The PHERAstar *FS* as well the POLARstar Omega offer the ability to perform the ADP-Glo™ Kinase Assay from Promega in a 384-well format using only 20 µL. Using either instrument, it is possible to choose to follow the reaction online using the kinetic mode or as an endpoint measurement.

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