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Fluorescence polarization based assay suitable for screening for H-Prostaglandin D Synthase inhibitors

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- The Prostaglandin D Synthase (hematopoietic-type) FP-Based Inhibitor Screening Assay Kit provides a robust and easy to use assay
- Both the CLARIOstar® and PHERAstar FS excel at detection of this fluorescence polarization based assay

Introduction

Prostaglandins are a group of lipid compounds that are involved in diverse effects in animal physiology. Prostaglandin D2 [PGD2] has been characterized for its role in asthma where its concentration has been shown to be 10 times higher in asthma patients leading to bronchial airway contraction after exposure to an allergen. For this and other reasons inhibitors are sought for the hematopoietic prostaglandin D synthase (H-PGDS) which catalyzes the final PGD2 biosynthesis step. Here we show the performance of Cayman Chemicals green FP-based inhibitor assay screening kit. The kit was tested with both the CLARIOstar and PHERAstar FS. Settings more suitable for high throughput screening (HTS) were also tested.

Assay Principle

The Prostaglandin D Synthase FP-Based Inhibitor Screening Assay provides a convenient one step assay. An H-PGDS inhibitor was conjugated to fluorescein and other inhibitors are discovered by their ability to displace this probe from binding with H-PGDS (Figure 1).

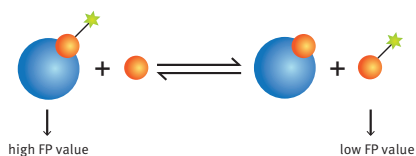


Fig. 1: H-PGDS Green FP Inhibitor Assay Principle.

When a fluorescein conjugated probe interacts with H-PGDS a high FP signal is detected. Inhibitors disrupt binding of the probe and the free probe exhibits a low FP signal.

In the absence of an effective inhibitor the probe will remain bound to H-PGDS thus reducing the rotational freedom of the probe, resulting in a high FP signal. Increasing concentrations of an effective inhibitor will lead to an increased amount of displaced probe. The displaced probe has an increased rotational freedom and therefore a lower FP signal.

This assay prevents the need for the traditional multistep assay that utilizes the highly unstable PGD2-precursor PGH2.

Materials & Methods

- Prostaglandin D Synthase (hematopoietic-type) FP-Based Inhibitor Screening Assay Kit – Green (Cayman Chemicals # 600007)
- PHERAstar FS and CLARIOstar microplate readers (BMG LABTECH)
- Inhibitors and additional reagents were purchased from commercial sources

To test kit function 16 point titration curves were prepared for 2 known H-PGDS inhibitors; HQL-79 (provided in the kit) and TFC 007. In addition a similar titration curve was prepared for AT 56 an inhibitor of lipocalin-type PGDS [L-PGDS]. For all 2-fold serial dilutions were made in DMSO.

Kit reagents were prepared according to kit instructions to create an assay cocktail. 47.5 µl of this cocktail was pipetted into the wells of a 384-well plate. This was followed by 2.5 µl of the appropriate inhibitor concentration or DMSO as a negative inhibition control. The final concentration of the 1st point in the titration curve was 2.5 mM for AT 56, 250 µM for HQL-79 and 25 µM for TFC 007. Following an incubation of 90 minutes at room temperature the plates were read using the following settings:

CLARIOstar settings:

Measurement method:	Fluorescence Polarization, End Point
Filter Settings:	482-16 / LP 504 / 530-40
Settling Time:	0.1
Number of flashes:	200
Focus and Gain:	Adjusted prior to measurement
Target mP:	200 mP set on DMSO control

PHERAstar FS settings:

Measurement method:	Fluorescence Polarization, End Point
Optic Module:	FP 485 520 520
Settling Time:	0.3 or 0.1
Number of flashes:	200 or 10
Focus and Gain:	Adjusted prior to measurement
Target mP:	200 mP set on DMSO control

Results & Discussion

We first wanted to confirm that the appropriate filters were selected by performing a spectral scan of the H-PGDS FP Fluorescent Probe – Green (Figure 2). The fluorescein conjugated probe behaves as expected, confirming the selection of CLARIOstar filters and of PHERAstar FS optic module.

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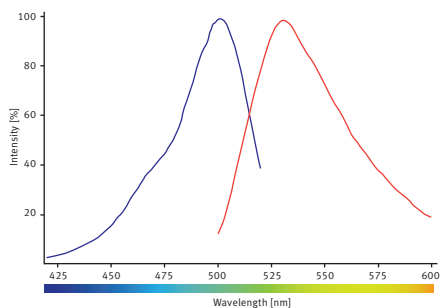


Fig. 2: H-PGDS FP Probe - Green Spectral Scan. Probe was scanned using a Fluorescence spectrum measurement. Excitation scan from 420-10 to 520-10 using 548-16 as the emission wavelength. Emission scan from 500-10 to 600-10 using 472-16 as the excitation wavelength.

The response to inhibitor treatments detected with the CLARIOstar is shown in Figure 3. The assay exhibits high quality based on the calculated Z' value of 0.84.

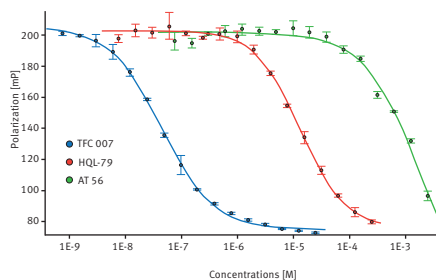


Fig. 3: Inhibitor titration curves on CLARIOstar. 4-parameter fit curves for TFC 007 (● $R^2=0.9991$), HQL-79 (● $R^2=0.998$) and AT 56 (● $R^2=0.984$).

From the 4-parameter fit curves we can determine the IC_{50} for inhibition of H-PGDS. TFC 007 exhibits an IC_{50} of 45 nM which corresponds well with the reported value of 83 nM¹. HQL-79 is reported to have an IC_{50} of 6 μ M² and in this experiment exhibited an IC_{50} of 13 μ M. AT 56 is an L-PDGS selective inhibitor and had previously been reported to have no effect on H-PGDS at concentrations as high as 100 μ M³. The results shown here predict an IC_{50} of 1.9 mM but would require further analysis at higher concentrations.

The PHERAstar FS provides similarly robust detection (Table 1). We therefore tested whether settings more conducive to HTS could be employed. Figure 4 shows results using 10 flashes which indicate that the PHERAstar FS maintains performance predicting similar IC_{50} values to those seen with the CLARIOstar.

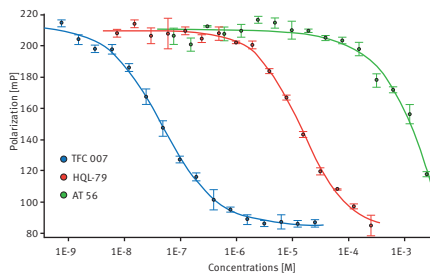


Fig. 4: Inhibitor titration curves on PHERAstar FS. 4-parameter fit curves for TFC 007 (● $R^2=0.998$, $IC_{50}=48$ nM), HQL-79 (● $R^2=0.997$, $IC_{50}=15$ μ M) and AT 56 (● $R^2=0.969$, $IC_{50}=1.25$ mM).

Table 1 shows that outstanding assay quality is maintained in the PHERAstar FS even though HTS settings are used.

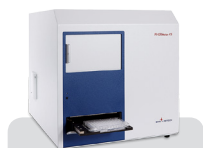
Flashes / Settling Time	Z'	384-well read time
200/0.3	0.82	7 minutes
10/0.1	0.74	1 minute 33 sec

Conclusion

Both the CLARIOstar and PHERAstar FS exhibit excellent assay quality when used to read Cayman's Prostaglandin D Synthase FP-Based Inhibitor Screening Assay. Furthermore the PHERAstar FS maintains this assay quality even when using settings more suitable for high throughput.

References

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- Artiaki K. et al. (2006) *J. Biol. Chem.* **281**(22) 15277-15286.
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PHERAstar® FS



CLARIOstar®