

Direct Quantification of PGD₂ by Fluorescence Polarization Immunoassay



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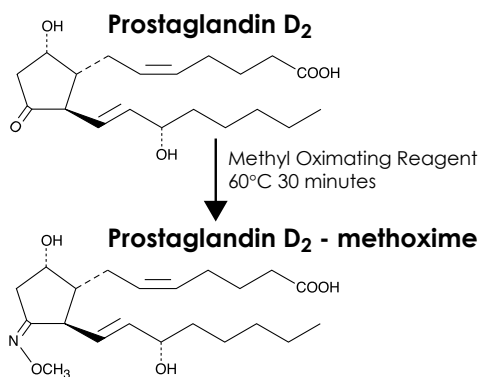
Direct Quantification of Prostaglandin D₂ by Fluorescence Polarization Immunoassay (FPIA)

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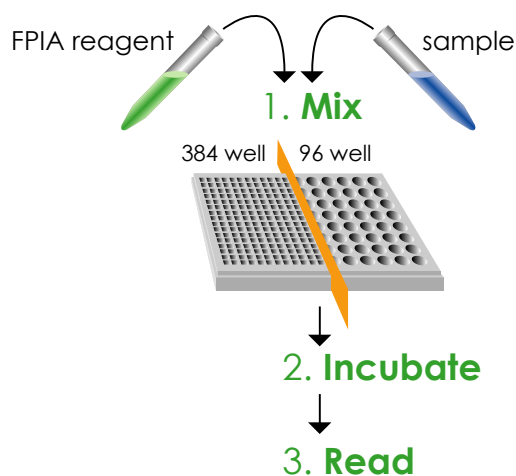
Abstract

Prostaglandin D₂ (PGD₂) is produced in large quantities by a hematopoietic-type PGD synthase in allergen-stimulated mast cells where it acts as a pro-inflammatory mediator in allergic reactions. PGD₂ is also biosynthesized in the brain by a lipocalin-type PGD₂ synthase where it acts in the CNS to promote sleep induction and lowering of body temperature. Current assays for the measurement of PGD₂ utilize a solid-phase EIA format that require multiple incubation and washing steps. Due to the inherent instability of PGD₂, conversion to a methoxylamine derivative is required prior to performing the EIA. We have developed a fluorescence polarization immunoassay (FPIA) for the rapid measurement of PGD₂ that does not require prior conversion to the methoxylamine compound. Fluorescence polarization (FP) assays are homogeneous, single-step assays ideal for high-throughput screening (HTS) applications. The PGD₂ FPIA uses a simple mix-and-read format in which a single reagent is added to the sample or standard and the assay is read after a 60 minute incubation. The assay is robust ($Z' = 0.74$), exhibits D100 mP over a range of 244 pg/ml to 1,000 ng/ml PGD₂, and has a detection limit of 700 pg/ml. Results from the measurement of PGD₂ in whole cell or cell lysate preparations show that the assay is applicable to screening COX or PGDS inhibitors.

Derivatization of PGD₂ for EIA



PGD₂ Measurement Using FPIA



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Methods and Results

Figure 1: Titration of PGD₂ Monoclonal Antibody and Assay Sensitivity at Various Antibody Concentrations

FP tracers were prepared by labeling PGD₂ with fluorescein. A PGD₂-specific monoclonal antibody was titrated to optimize the dynamic range and sensitivity of the assay.

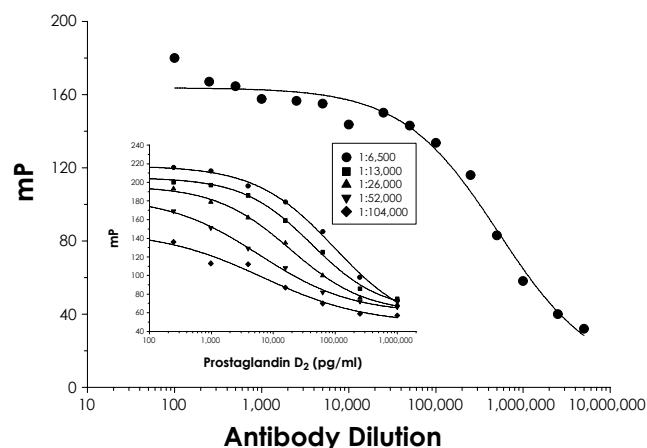


Figure 2: PGD₂ FPIA Standard Curve

The PGD₂ FPIA Standard Curve prepared in FPIA buffer shows a change of ~100 mP and a sensitivity of approximately 700 pg/ml (3s from zero PGD₂).

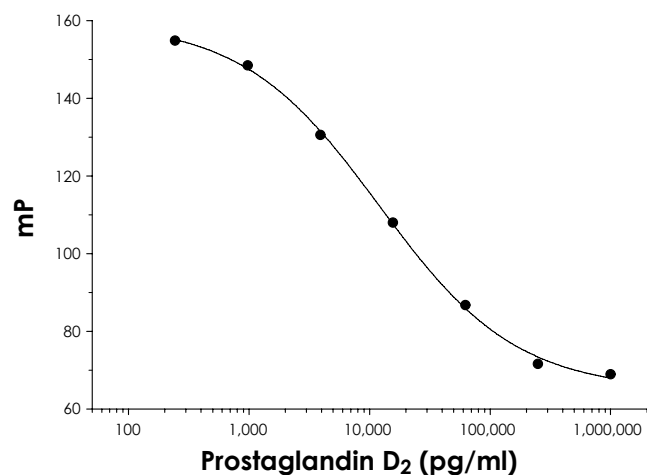


Figure 3: Time-Dependent Change in PGD₂ FPIA Standard Curves

PGD₂ standard curves were prepared in assay buffer and FP was measured at the indicated times. Reproducible curves are obtained after a 60 minute incubation.

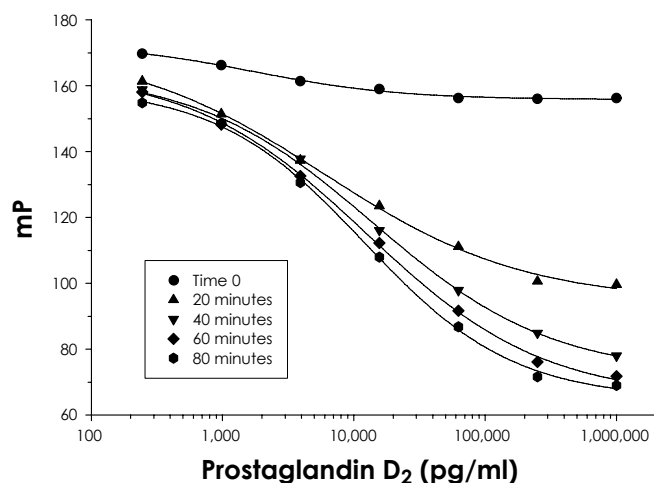
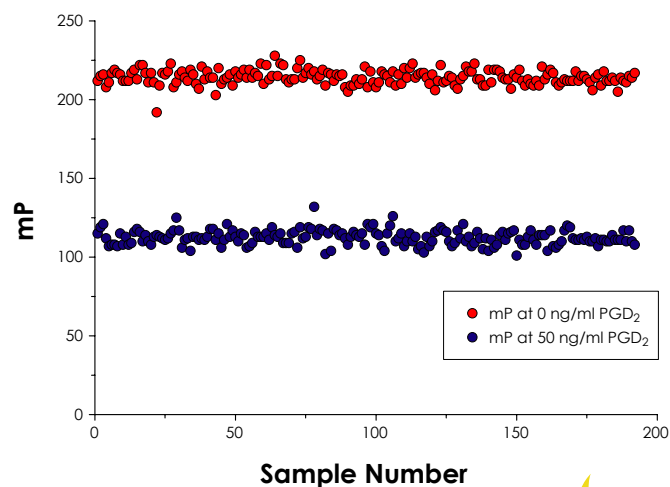


Figure 4: Z-Factor Determination

Z-Factor for the PGD₂-FPIA was determined by measuring mP in four 96 well plates. Each plate had 48 negative control wells (no PGD₂) and 48 positive control wells containing 50 ng/ml PGD₂. The Z-Factor was determined to be 0.74, indicating the assay is robust.



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Figure 5: Simultaneous measurement of PGD₂ and PGE₂

PGD₂ and PGE₂ standard curves were prepared separately (control) and in the same well (duplex). Polarization was measured with the appropriate filters (fluorescein – ex: 485 nm, em: 530 nm, dichroic: 505 nm • rhodamine – ex: 560 nm, em: 645 nm, dichroic: 595 nm)

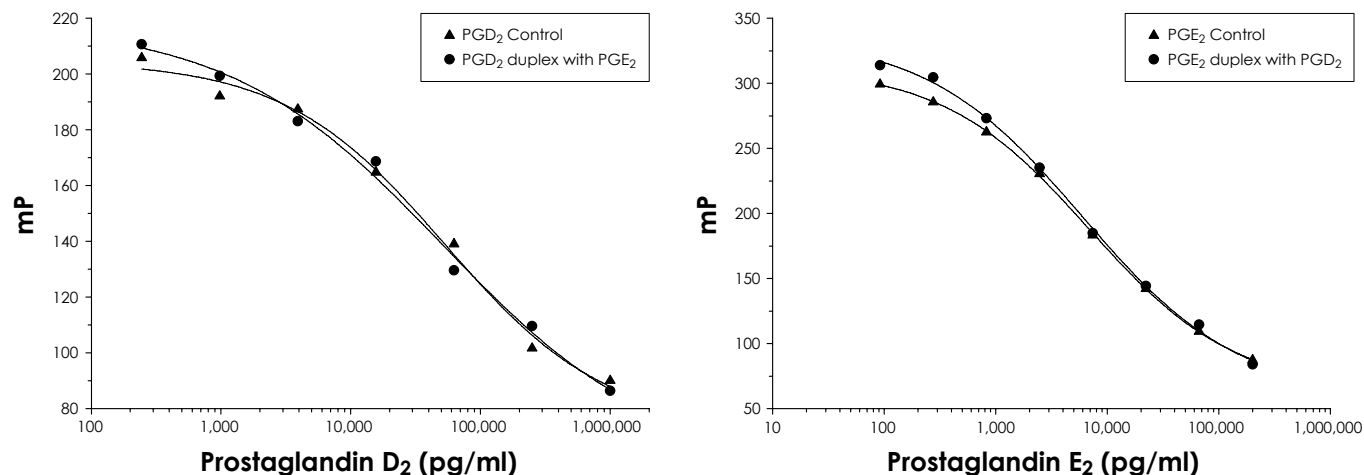
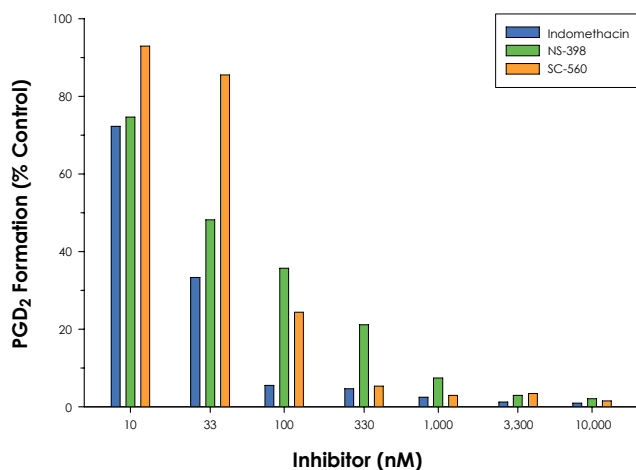


Figure 6: Assay of PGD₂ in RAW 264.7 Cells

LPS-induced RAW 264.7 cells were washed 1x with PBS. The cells were incubated for 30 minutes with inhibitor at 37°C. The PBS was replaced with fresh PBS containing 0.1% BSA (Panvera P-2489), 10 µM arachidonic acid and inhibitor for 30 minutes.



PGD₂ FPIA Cross-Reactivity

Prostaglandin D ₂	100%
Prostaglandin F _{2a}	0.79%
6-keto Prostaglandin F _{1a}	0.33%
Tetranor PGEM	0.03%
Thromboxane B ₂	1.34%
Prostaglandin E ₂	0.52%
Arachidonic Acid	0.14%

Conclusions

1. The PGD₂ FPIA is a direct homogeneous assay which does not require derivatization of PGD₂ as required in typical EIA formats and is suitable for HTS purposes
2. The assay is characterized by a broad standard curve and a Z-factor of 0.74. These properties allow the accurate measurement of PGD₂ while minimizing the need for multiple sample dilutions.
3. Fluorescein-labeled PGD₂ absorbs in the spectral region where library compounds may also absorb and is prone to interference by sample matrix components. Modifications of the assay are currently underway to utilize a red-shifted tracer, such as rhodamine. However, a primary advantage of the current assay is the ability to duplex with the PGE₂ FPIA-Red for measurement of both eicosanoids from the same sample.



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