

Fluorescence Polarization Immunoassay (FPIA) for PGE₂ Based on a Rhodamine Fluorophore

1180 E. Ellsworth Road • Ann Arbor, MI 48108 • Toll Free 800.364.9897 • www.caymanchem.com



Fluorescence Polarization Immunoassay (FPIA) for Prostaglandin E₂ Based on a Rhodamine Fluorophore

Dan Tew, Adam Uzieblo, Michelle Stanton, Elizabeth Meade, Jeff Johnson, and Kirk Maxey

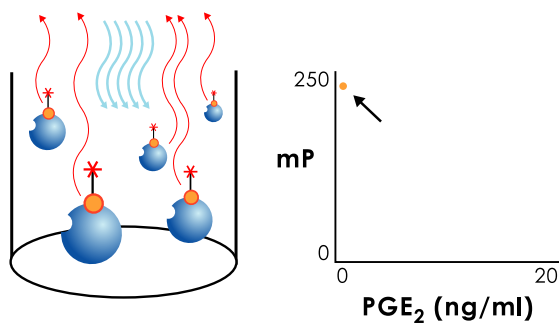
Abstract

Prostaglandin E₂ (PGE₂) is a primary prostaglandin formed from the coupled metabolism of arachidonic acid by the cyclooxygenases (COX-1 and COX-2) and prostaglandin E synthase. Its activity influences inflammation, fertility and parturition, gastric mucosal integrity, and immune modulation. Most immunoassays for the measurement of PGE₂ utilize a solid-phase format that requires multiple incubation and washing steps. We have developed a rhodamine-based fluorescence polarization immunoassay (FPIA) for the rapid measurement of PGE₂.

Fluorescence polarization (FP) assays are homogeneous, single-step assays ideal for high-throughput screening (HTS) applications. The PGE₂ FPIA-Red uses a simple mix-and-read format in which a single reagent is added to the sample/standard and the assay is read after a 60 minute incubation. The assay is robust ($Z' = 0.69$), exhibits D200 mP over a range of 91 pg/ml to 200 ng/ml PGE₂, and has a sensitivity of 210 pg/ml. Use of the red-shifted rhodamine label reduces interference from most sample matrices thereby making the assay applicable to the identification of COX-1/-2 or PGE Synthase inhibitors using whole cells, cell lysates, plasma, or recombinant enzyme preparations.

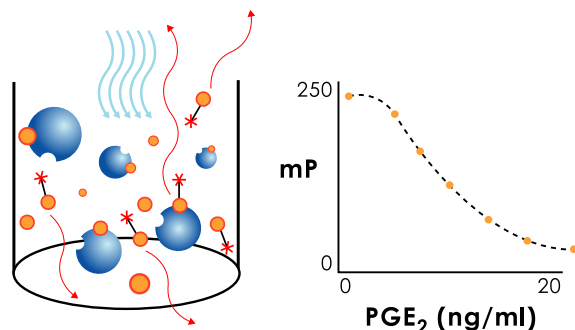
Low free PGE₂ concentration:

PGE₂-Rhodamine bound to the antibody rotates slowly and exhibits high polarization



High free PGE₂ concentration:

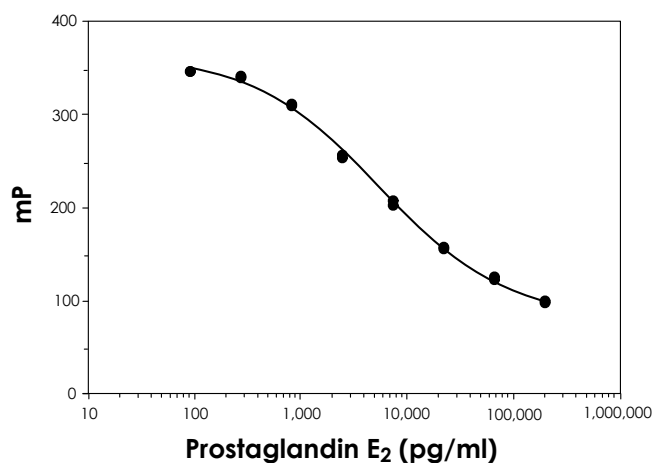
Excess free PGE₂ displaces PGE₂-Rhodamine which rotates rapidly and exhibits low polarization



Methods and Results

Figure 1: PGE₂ FPIA-Red Standard Curve

FP tracers were prepared by labeling PGE₂ with rhodamine. A PGE₂-specific monoclonal antibody and the tracer were titrated to optimize the dynamic range and sensitivity of the assay. The PGE₂ FPIA Standard Curve in buffer shows a sensitivity of approximately 210 pg/ml (3s from zero PGE₂).



800.364.9897 www.caymanchem.com/FPIA

Cayman
CHEMICAL

Fluorescence Polarization Immunoassay (FPIA) for PGE₂ Based on a Rhodamine Fluorophore

1180 E. Ellsworth Road • Ann Arbor, MI 48108 • Toll Free 800.364.9897 • www.caymanchem.com



Figure 2: Z-factor Determination

Z-Factor for the PGE₂ FPIA was determined by measuring mP in four 96 well plates. Each plate had 48 negative control wells (no PGE₂) and 48 positive control wells containing 5000 pg/ml PGE₂. The Z-Factor was determined to be 0.69, indicating a robust assay.

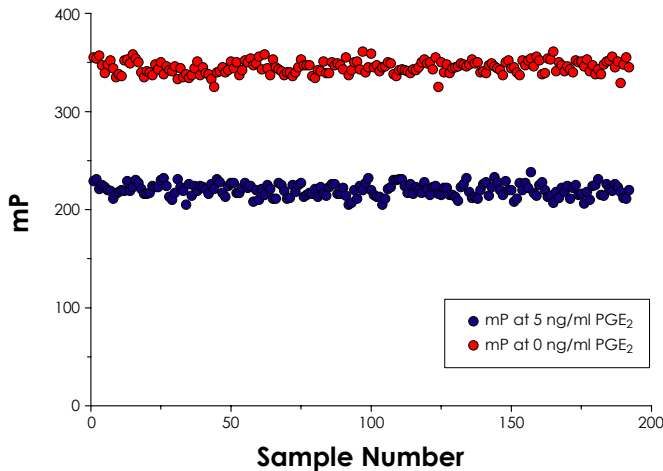


Figure 3: PGE₂ FPIA in Ethanol-Extracted Plasma

Human plasma was deproteinated using 4 volumes of ethanol. The ethanol extract was dried and reconstituted in FPIA buffer. Standard curves were performed in various dilutions of the extract.

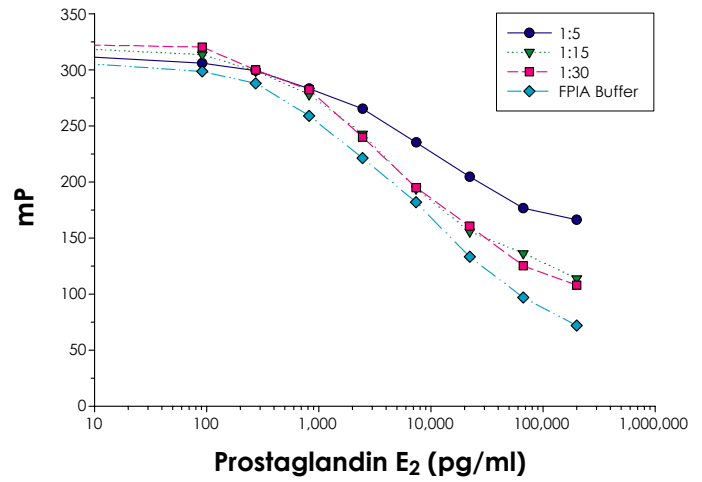
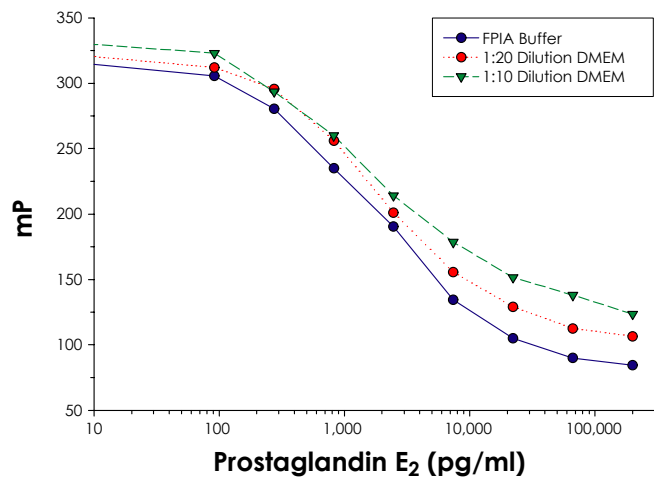
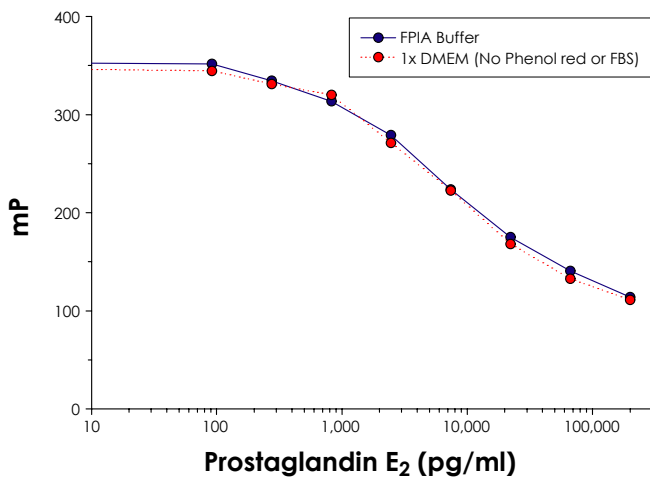


Figure 4: PGE₂ FPIA Standard Curves in Culture Media

Left: Comparison of PGE₂ FPIA standard curves in phenol red-free DMEM

Right: PGE₂ Standard curves in various dilutions of DMEM containing phenol red and 10% FBS



Fluorescence Polarization Immunoassay (FPIA) for PGE₂ Based on a Rhodamine Fluorophore

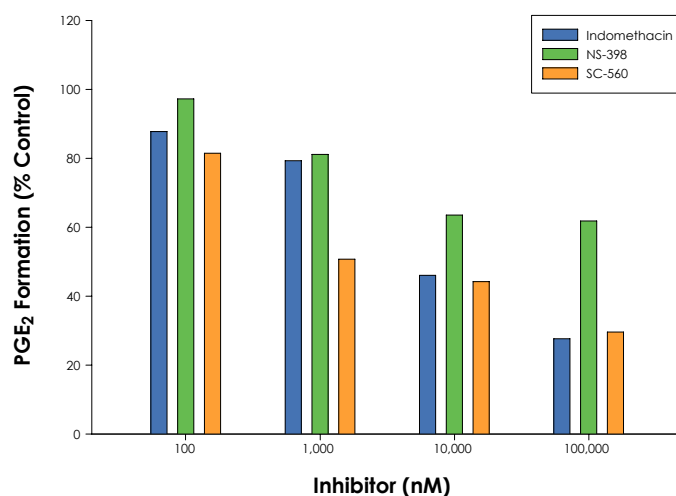
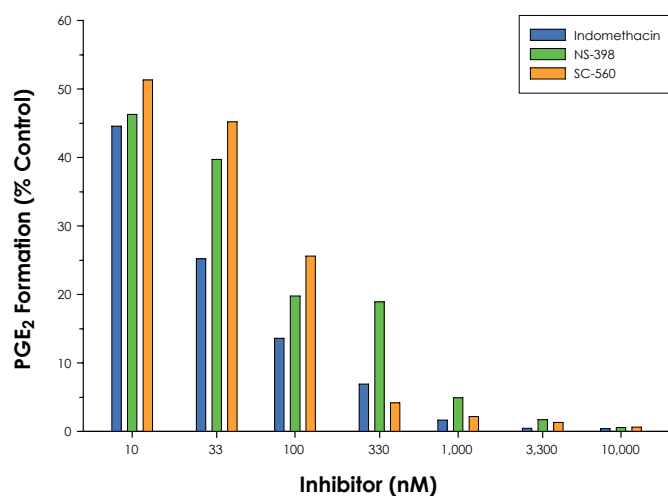
1180 E. Ellsworth Road • Ann Arbor, MI 48108 • Toll Free 800.364.9897 • www.caymanchem.com



Figure 5: Inhibition of PGE₂ Formation in RAW 264.7 Cells or Cell Lysates

Whole Cells (Left): 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.

Cell lysates (Right): Lysates from LPS-induced RAW 264.7 cells were incubated with inhibitor for 2 minutes. Reactions were initiated with 20 μM arachidonic acid. After 5 minutes, reactions were terminated by the addition of 1 M sodium citrate, pH 4.0, and FeCl₂. Samples were neutralized and assayed directly by FPIA.



PGE₂ FPIA Cross-Reactivity

Prostaglandin E ₂	100%
Prostaglandin E ₁	100%
Prostaglandin E ₂ Ethanolamide	100%
Prostaglandin E ₃	85%
Sulprostone	9.0%
6-keto Prostaglandin F _{1a}	2.9%
8-iso Prostaglandin F _{2a}	0.09%
Prostaglandin D ₂	<0.01%
8-iso Prostaglandin E ₂	<0.01%

Conclusions

1. The PGE₂ FPIA-Red is a homogeneous assay suit able for HTS applications. It can be used for COX or PGES inhibitor screening or for other applications which utilize PGE₂ as the primary readout.
2. The assay is characterized by a broad standard curve with a Z-factor of 0.69. These characteristics provide a means for the accurate measurement of PGE₂ without the need for multiple sample dilutions.
3. The use of a rhodamine tracer in this assay allows measurements of PGE₂ in both cell culture media and plasma samples. Care should be still be exercised during sample analysis to ensure the utilization of proper controls as minor matrix effects are evident.



800.364.9897 www.caymanchem.com/FPIA

