> PGE_2 FPIA kit

Catalog # ADI-920-001

Fluorescence Polarization Immunoassay Kit
For use with cell supernatants

Reagents require separate storage conditions

Check our website for additional protocols, technical notes and FAQs

For proper performance, use the insert provided with each individual kit received

Table of Contents

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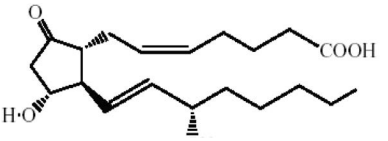
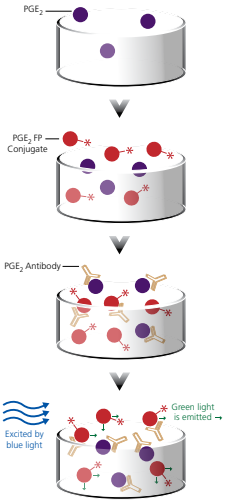
Introduction

The PGE_2 FPIA kit is a complete kit for the quantitative determination of PGE_2 in buffers and culture media.

Prostaglandin E_2 (PGE_2) is formed in a variety of cells from PGH_2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase[1-4]. PGE_2 has been shown to have a number of biological actions, including vasodilation[5], both anti- and proinflammatory action[6,7], modulation of sleep/wake cycles[8], and facilitation of the replication of human immunodeficiency virus[9]. It elevates cAMP levels[10], stimulates bone resorption[11], and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics[12].

Prostaglandin E_2





Principle

1. Samples and standards are added to uncoated wells.

2. A solution of PGE_2 covalently conjugated to fluorescein is then added to the wells.

3. A solution of monoclonal antibody to PGE_2 is next added. This binds, in a competitive manner, the PGE_2 in the standard, sample or conjugate.

4. The plate is incubated at room temperature for at least 30 minutes. The FP signal is stable for at least 20 hours.

5. The plate is then read at 520 - 535 nm, with excitation at 485 nm. The amount of signal is inversely proportional to the concentration of PGE_2 in the standards or samples.

Materials Supplied

1. **PGE_2 FPIA Antibody**
   8 mL, Catalog No. 80-1437
   A solution of monoclonal antibody to PGE_2.

2. **Assay Buffer 1 Concentrate**
   125 mL, Catalog No. 80-1435
   Tris buffered saline containing proteins and sodium azide as preservative.

3. **PGE_2 FPIA Conjugate Concentrate**
   0.1 mL, Catalog No. 80-1438
   A solution of fluorescein conjugated to PGE_2.

4. **PGE_2 Standard**
   0.5 mL, Catalog No. 80-1436
   A solution of 1,000,000 pg/mL PGE_2.

Wear gloves while using the conjugate.

Protect conjugate from light.

Reagents require separate storage conditions.

Storage

All components of this kit, **except the PGE_2 FPIA Conjugate Concentrate and PGE_2 Standard**, are stable at 4°C until the kit's expiration date. The PGE_2 FPIA Conjugate Concentrate and PGE_2 Standard **must** be stored at -20°C.

Materials Needed but Not Supplied

1. Solid black uncoated low-binding microtiter plate.
2. Foil microtiter plate sealer, if desired.
3. Fluorescense polarization detector, such as a Dynex Triad Multimode, capable of reading emissions at 520 - 535 nm, with excitation at 485 nm.

Reagent Preparation

1. **Assay Buffer**
   Prepare the assay buffer by diluting 100 mL of the supplied Assay Buffer 1 Concentrate with 900 mL of deionized water. This can be stored at room temperature until the kit's expiration, or for 3 months, whichever is earlier. Use the diluted assay buffer for the entire assay.

Protect conjugate from light.

2. **PGE_2 Conjugate**
   Count the total number of wells that will recieve conjugate. Use the following formula to calculate the volume of PGE_2 Conjugate Concentrate and the assay buffer needed to prepare PGE_2 conjugate.

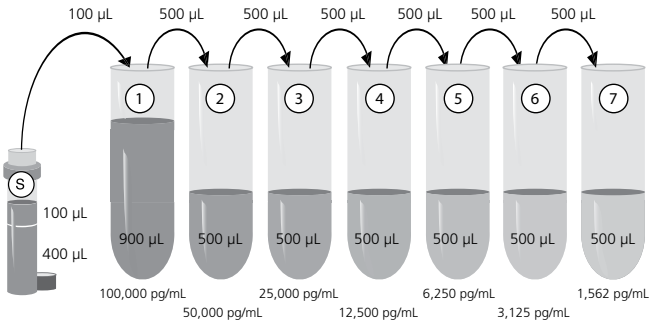
   A. (Number of wells + 1) x 0.05 mL / well = Volume of assay buffer needed. Increase the calculated volume to the next whole milliliter.

   B. (Volume from part A) x 10 µL / mL = Volume of PGE_2 Conjugate Concentrate needed.

   Pipet the volume of assay buffer from part A into an amber container. From this volume, remove the volume calculated in part B. Add the calculated PGE_2 Conjugate Concentrate to the assay buffer. Vortex thoroughly and use.

Do not store prepared PGE_2 conjugate. Make fresh for each assay

4. **PGE_2 Standards**



Glass tubes must be used for standard preparation

Allow the 1,000,000 pg/mL standard stock to warm to room temperature. Label seven 12 x 75 tubes #1 through #7. Pipet 900 µL of the assay buffer into tube #1. Pipet 500 µL of the assay buffer into tubes #2 through #7. Add 100 µL of the 1,000,000 pg/mL PGE_2 Standard into tube #1 and vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #7. The concentrations of PGE_2 in the tubes are labeled above.

Diluted standards should be used within 60 minutes of preparation.

Sample Handling

The FPIA is compatible with PGE_2 samples in defined buffers and cell culture media. Samples diluted sufficiently into the assay buffer can be read directly from the standard curve. Samples containing some organic solvents or inherently fluorescing materials may interfere with the assay. Please refer to the Interferences section on page 10 for details.

Sample Recoveries

PGE_2 standard was spiked into the following buffers, which were already diluted with the assay buffer, and measured in the kit. The results are shown below. These are the minimum dilutions required to remove the matrix interference of these solutions.

Sample	Recovery	Recommended Dilution
Hanks Buffered Saline Solution	108%	≥ neat
RPMI-1640 without phenol red	95%	≥ 1:4
RPMI-1640 with phenol red, sodium pyruvate and Essential Amino Acids	100%	≥ 1:16
RPMI-1640 with phenol red, 10% FBS and antibiotics	105%	≥ 1:32

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards, controls and samples should be run in duplicate.

The following procedure was used to generate all performance data cited in this publication.

1. Pipet 150 µL of assay buffer into the Total Fluorescence (TF) wells.
2. Pipet 100 µL of assay buffer into the Bo (0 pg/mL) wells.
3. Pipet 100 µL of Standards #1 through #7 to the bottom of the appropriate wells.
4. Pipet 100 µL of the samples to the bottom of the appropriate wells.
5. Add 50 µL of the conjugate into each well.
6. Add 50 µL of antibody into each well, except the TF wells.
7. Seal the plate with a foil plate sealer. Incubate the plate for at least 30 minutes at room temperature. The FP signal is stable for at least 20 hours.
8. Read plate on a suitable fluorescence polarization detector at 520 - 535 nm emission, with excitation at 485 nm, using the appropriate settings for that instrument.

Calculation of Results

Several options are available for the calculation of the concentration of PGE_2 in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the binding for each standard and sample as a percentage of the maximum binding (Bo), using the following formula:

$$\textbf{Percent Bound} = \frac{\textbf{Average mP}}{\textbf{Average Bo}} \times 100$$

2. Plot the Percent Bound for each standard versus PGE_2 concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

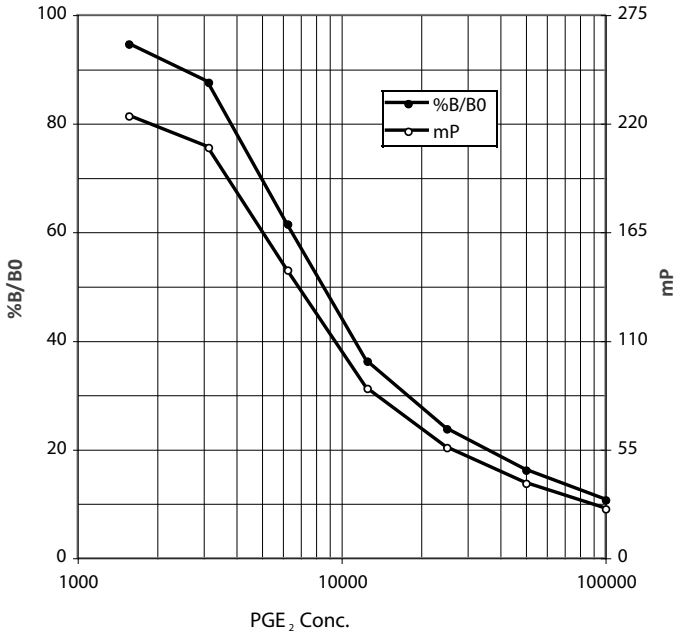
Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a higher dilution.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net mP	Percent Bound	PGE_2 (pg/mL)
S1	25	10.7%	**100,000**
S2	38	16.2%	**50,000**
S3	56	23.8%	**25,000**
S4	86	36.3%	**12,500**
S5	146	61.5%	**6,250**
S6	208	87.6%	**3,125**
S7	224	94.7%	**1,562**
Unknown 1	136	57.4%	**7,204**
Unknown 2	48	20.3%	**27,803**
Bo	237	100%	**0**



Performance Characteristics

Specificity

For detailed cross-reactivity protocol see our website .

The cross reactivities for a number of related compounds were determined by diluting the compounds in the kit assay buffer at concentrations from 1,000,000 to 1,000 pg/mL. These samples were then measured in the PGE_2 FPIA, and the measured PGE_2 concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of the cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
PGE_2	100%
PGE_1	100%
PGD_2	11%
$PGF_{2\alpha}$	2.5%
6-keto-$PGF_{1\alpha}$	2.3%
PGB_2	1.8%
PGI_2	0.9%
TXB_2	0.3%
Arachidonic acid	<0.1%
Dihomo-γ-linolenic acid	<0.1%

Z-Factor

The Z-Factor is a dimensionless statistic that reflects the dynamic signal range and variation of an assay. This provides a useful parameter to evaluate the robust quality of a given assay[14]. The following formula was used:

$$\text{Z-Factor} = 1 - \left[\frac{(3 \times 1SD \text{ of Positive}) + (3 \times 1SD \text{ of Negative})}{\text{Positive} - \text{Negative}}\right]$$

The Z-Factor of the assay was determined to be 0.88.

Sensitivity

The sensitivity of the assay, defined as the concentration of PGE_2 measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 684 pg/mL.

Linearity

A buffer sample containing PGE_2 was serially diluted 1:2 in the kit assay buffer and measured in the assay. The results are shown in the table below.

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Neat	---	76,035	---
1:2	38,017	31,610	83%
1:4	19,009	19,701	104%
1:8	9,504	11,713	123%
1:16	4,752	4,228	89%
1:32	2,376	2,329	98%

Precision

Intra-assay precision was determined by assaying 24 replicates of three buffer controls containing PGE_2 in a single assay.

pg/mL	%CV
24,142	21
16,629	13
5,392	8.3

Inter-assay precision was determined by measuring buffer controls of varying PGE_2 concentrations in multiple assays over several days.

pg/mL	%CV
24,103	11.4
15,598	10.7
6,574	12.7

Interferences

PGE_2 standard was spiked into the following solutions, which were already diluted with the assay buffer, and measured in the kit. The results were as follows:

Sample	Recovery	Recommended Percent
Methanol	107%	≤ 10%
Acetonitrile	109%	≤ 5%
2-propanol	98%	≤ 2.5%
Ethanol	106%	≤ 2.5%
DMF	104%	≤ 1.3%
DMSO	102%	≤ 1.3%

References

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Notes

MSDS (Material Safety Data Sheet) available online

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