Prostaglandin E_2 FPIA Kit - Red

Item No. 10004517

TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Precautions
4 If You Have Problems
4 Storage and Stability
4 Materials Needed but Not Supplied

INTRODUCTION
5 Background
5 About This Assay
7 Introduction to FPIA

PRE-ASSAY PREPARATION
11 Buffer Preparation
11 Sample Preparation

ASSAY PROTOCOL
13 Preparation of Assay-Specific Reagents
15 Performing the Assay
17 Testing for Interference

ANALYSIS
18 Calculations
19 Performance Characteristics

RESOURCES
23 Troubleshooting
23 Additional Reading
24 References
25 Related Products
26 Warranty and Limitation of Remedy
27 Plate Template
28 Notes

GENERAL INFORMATION

Materials Supplied

Item Number	Item	384 wells Quantity/Size	1,920 wells Quantity/Size
10004565	Prostaglandin E_2 FPIA Reagent - Red	1 vial/400 dtn	1 vial/2,000 dtn
10004566	Prostaglandin E_2 FPIA Standard - Red	1 vial	1 vial
400501	FPIA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
10005371	384-Well Solid Plate (black; non-binding)	1 plate	5 plates
400023	Foil Plate Cover	1 cover	5 covers

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with the Cayman Prostaglandin E_2 FPIA - Red Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
E-Mail:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of fluorescence polarization measurements using rhodamine as the fluorophore.
2. Adjustable pipettes and a repeat pipettor.

INTRODUCTION

Background

Prostaglandin E_2 (PGE_2) is a primary product of arachidonic acid metabolism in many cells. Like most eicosanoids, it does not exist preformed in any cellular reservoir. When cells are activated or exogenous free arachidonate is supplied, PGE_2 is synthesized *de novo* and released into the extracellular space. *In vivo*, PGE_2 is rapidly converted to an inactive metabolite (13,14-dihydro-15-keto PGE_2) by the prostaglandin 15-dehydrogenase (15-OH PGDH) pathway[1,2] (see Figure 1, page 6). The half-life of PGE_2 in the circulatory system is approximately 30 seconds and normal plasma levels are 3-12 pg/ml.[3] Cultured cells secrete PGE_2 into the medium, where it accumulates to levels as high as several ng/ml without appreciable metabolism.

About This Assay

Cayman's PGE_2 FPIA - Red Assay has been validated for use with samples obtained from culture medium in whole cell experiments and *in vitro* assay systems. These samples can be added directly to the well (a minimum dilution may be required), mixed with the FPIA Reagent, and the PGE_2 concentration determined in 60-90 minutes. Further dilution may be desirable to bring the concentration of the sample onto the most linear part of the standard curve, between 300-25,000 pg/ml. Normal plasma PGE_2 concentrations are too low (<20 pg/ml) to be easily measured by FPIA; we suggest that our high sensitivity PGE_2 EIA (Item No. 514010) be used for these samples. Highly metabolized samples such as urine contain mostly 13,14-dihydro-15-keto PGE and PGA species, and are suitable for analysis with our PGE_2 Metabolite EIA Kit (Item No. 514531).

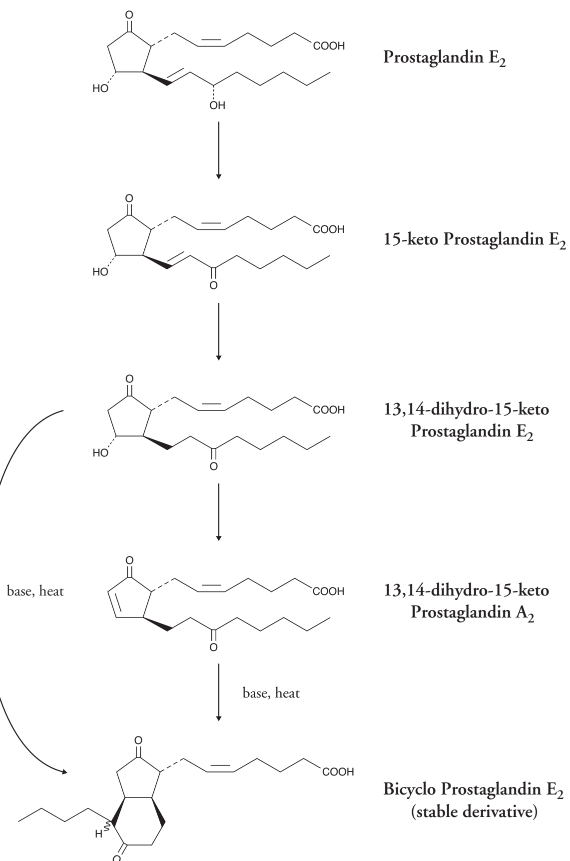


Figure 1. The metabolism of PGE_2

Introduction to FPIA

Fluorescence polarization (FP) assays are homogeneous, single-step assays ideally suited for high-throughput screening (HTS) of large numbers of samples. All FP assays employ a large molecular species, or binding partner (BP) in conjunction with a small, low molecular weight fluorescent analyte (FA). When the large BP molecule is an antibody, the assay is referred to as a fluorescence polarization immunoassay (FPIA).

Fluorescence is by definition the ability of a molecule to absorb the energy of an incoming (excitation) photon and then re-emit most of this energy as a new, slightly less energetic (emission) photon.

A small fluorescent molecule will rotate appreciably during the very small interval of time between absorption of a photon and emission of the fluorescence photon.

If the excitation light is polarized, this rotation will result in complete randomization of the plane of the emitted light. Thus, small fluorescent molecules depolarize an excitation pulse of polarized light (see well #1 in Figure 2, below).

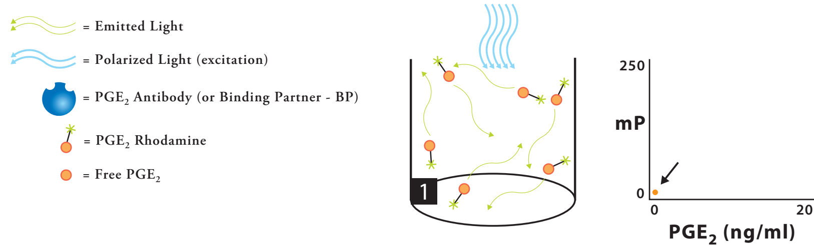


Figure 2. Small fluorescent molecules rotate rapidly and depolarize the excitation light

Large fluorescent molecules (MW >100,000) do not rotate appreciably in the same small interval of time. They will therefore emit light that retains some of the polarization of the polarized excitation light. This polarization is quantified in milli-polarization units, or mP. A fluorescence polarization reader is required to make this measurement.

When a small fluorescent molecule becomes tightly bound to a large one, as in the binding of the PGE_2 antibody to PGE_2 rhodamine, the rotational speed of the small molecule is abruptly reduced to that of the entire complex as a whole (see well #2 in Figure 3, below).

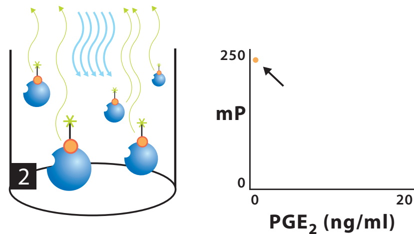


Figure 3. Large fluorescent molecules rotate slowly and emit light that retains some of the polarization of the excitation light

Therefore, PGE_2-rhodamine bound to its antibody represents a large fluorescent molecule, which exhibits a high degree of FP. A microplate well filled with the PGE_2-rhodamine:antibody complex will give a high FP reading. The PGE_2 FPIA is based on the competition of free PGE_2 in the samples or standards for the high affinity binding site of a PGE_2 monoclonal antibody occupied by PGE_2-rhodamine. Addition of a small amount of unlabeled PGE_2 will result in a competition between the unlabeled PGE_2 and the PGE_2-rhodamine for the antibody (see Figure 4 below).

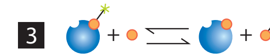


Figure 4. Competition between PGE_2 and the PGE_2-rhodamine tracer for the PGE_2 Monoclonal Antibody

Some of the fluorescent rhodamine-conjugate will be released from the antibody, and will resume its intrinsic, rapid rate of rotation. This will cause a detectable loss of FP in the well (see well #4 in Figure 5, below).

The addition of a large amount of PGE_2 (50-100 ng/ml) will result in a much larger reduction in the mP of the well (see well #5 in Figure 5, below). Plotting mP *versus* PGE_2 allows the construction of a standard curve with a broad dynamic range. This is similar to, but not strictly analagous to, the sigmoidal dose-response curve in a traditional solid phase EIA.

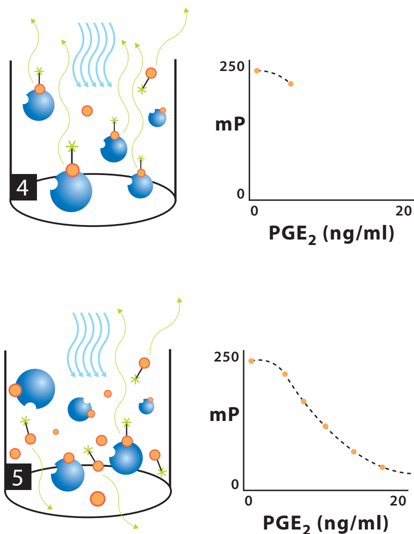


Figure 5. Loss of fluorescence polarization following the addition of increasing amounts of PGE_2 to the PGE_2 FPIA

Cayman's PGE_2 FPIA - Red gives accurate quantitation of PGE_2 in the range of 400 pg/ml - 40 ng/ml within 60 minutes. Any samples falling outside this range should be diluted or concentrated prior to analysis.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all FPIA reagents and buffers must be deionized and free of trace organic contaminants ("UltraPure"). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for FPIA. UltraPure water may be purchased from Cayman Chemical (Item No. 400000).

Buffer Preparation

Dilute the contents of one vial of FPIA Buffer Concentrate (10X) (Item No. 400501) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.* Store the buffer at 4°C; it will be stable for approximately one month.

Sample Preparation

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Cultured cells

Samples containing culture medium cannot be used directly in the assay with the following exceptions. Phenol red-free DMEM can be used in the assay directly without dilution. Phenol red-free DMEM with 10% FBS can be used in the assay at a minimum of 1:5 dilution and phenol red-free DMEM with 20% FBS can be used in this assay at a minimum of 1:10 dilution. Variations of this methodology may be employed, provided care is taken to assess potential interference from the use of any buffers utilized in the assay. See **Testing for Interference** on page 17.

In vitro Enzyme Assays

The PGE_2 FPIA is well suited for analysis of PGE_2 samples from *in vitro* assay systems (*i.e.*, microsomal or purified preparations of COX and PGE synthase or analogous expression systems). The buffer utilized in the assay system should be checked for interference in the FPIA as described below (See **Testing for Interference** on page 17).

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

PGE_2 FPIA Reagent - Red

Reconstitute the PGE_2 FPIA Reagent - Red as follows:

a. **400 dtn PGE_2 FPIA Reagent - Red (384-well kit; Item No. 10004565):** Reconstitute with 12 ml FPIA Buffer.

OR

b. **2,000 dtn PGE_2 FPIA Reagent - Red (1,920-well kit; Item No. 10004565):** Reconstitute with 60 ml FPIA Buffer.

NOTE: 12 ml of reconstituted reagent is enough for either a standard 96 or 384-well plate. Allow the reagent to equilibrate to room temperature prior to use. Store unused PGE_2 FPIA Reagent - Red at 4°C and use within 72 hours.

PGE_2 FPIA Standard - Red

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the PGE_2 FPIA Standard - Red (Item No. 10004566) into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 2,000 ng/ml.

To prepare the standard for use in the FPIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl FPIA Buffer to tube #1 and 1,000 µl FPIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (2,000 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.

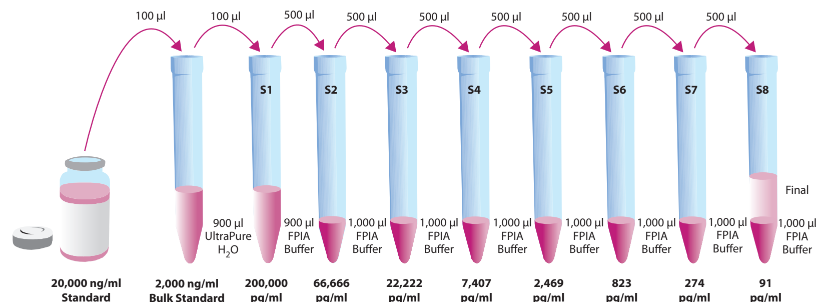


Figure 6. Preparation of the PGE_2 standards

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Do not expose the pipette tip to the reagent(s) already in the well.
- Avoid introducing bubbles into the wells.

Follow the steps below to accurately measure mP in the assay. Allow all reagents to equilibrate to room temperature prior to performing the assay. *NOTE: Volumes shown are for a 384-well plate format. For a 96-well plate format, use 100 µl of standard, sample, or FPIA Buffer and 100 µl of FPIA Reagent.*

1. PGE_2 FPIA Standard - Red

a. Add 25 µl from tube #8 to both of the lowest standard wells (S8). Add 25 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

b. Add 25 µl of FPIA Buffer to a minimum of two wells. These wells will serve as the maximum mP (mP_{max}) wells and will quantify the maximum signal possible in the absence of any unlabeled PGE_2.

2. Samples

Add 25 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed at least in duplicate (triplicate recommended).

Optional Step

Read and store the emission for each well in both parallel and perpendicular modes (see details on reading the plate in step 5, on page 16). These values can be subtracted from the corresponding values in the presence of the PGE_2 FPIA Reagent to eliminate any background fluorescence in the sample, thereby increasing the accuracy of the assay.

3. PGE_2 FPIA Reagent - Red

Add 25 µl of FPIA Reagent to each of the wells on the plate.

4. Incubation of the Plate

Cover the plate with the plate cover supplied in the kit and incubate for 60-90 minutes at room temperature on an orbital shaker. The FP signal is stable for at least three hours.

5. Reading the Plate

Read the plate(s) with excitation and emission wavelengths of 560 nm and 645 nm, respectively. The plate reader used at Cayman Chemical employs a dichroic filter at 590 nm. Some instruments may not utilize this type of filter. The excitation polarizer is set in the horizontal position, and the emission polarizer is dynamic. The measurements are taken with the z-height set to the middle of the well and the G-factor set to one.

Testing for Interference

The types of buffers and samples potentially utilized in this assay can vary widely. To test for interference in the sample matrix perform the following test. Prepare 2-fold serial dilutions of the sample matrix in the FPIA Buffer beginning at a 1:2 dilution (*i.e.*, make dilutions of 2, 4, 8, 16, 32, 64, *etc.*). Add reagents to the plate as described above and measure the mP_{max} in the presence of the undiluted matrix and for each of the dilutions. As a control, measure mP using only FPIA Buffer with the FPIA Reagent - Red.

If there is no interference from the sample matrix, the mP values at all dilutions will be at the mP_{max} level. Interference will cause a reduction in mP that will diminish as dilutions of the sample matrix increase. A minimum dilution required for removal of the interference can be easily determined using a plot of mP *versus* dilution. It may be possible to perform the assay at suboptimal sample matrix dilutions, provided the standard and all samples contain the same amount of sample matrix and therefore exhibit the same level of signal suppression.

ANALYSIS

Cayman has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/fpia) for more information or to obtain a free copy of this convenient data analysis tool.

Calculations

Fluorescence polarization of a molecule is defined as:

$$\text{Polarization (mP)} = 1{,}000 \times \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + I_{\perp})} \qquad \text{Range: 0 to 500 mP}$$

A plot of mP *versus* PGE_2 concentration on semi-log axes results in a sigmoidal dose-response curve typical of competitive binding assays. This data can be fit to a 4-parameter logistic equation as shown in Figure 7 (see page 21).

A second method of data analysis uses a log-logit plot. The log-logit method is a transformation based on the following equation:

$$\text{logit}(y) = \ln[y/(1-y)] \qquad \text{where } y = (mP_{\text{standard or sample}}/mP_{max})$$

The logit transformation reduces the sigmoidal curve of mP *versus* log concentration to a straight line of logit $mP_{standard}/mP_{max}$ *versus* PGE_2 on semi-log axes. The curve is completely described by the y-intercept and the slope of the line, which can be used to calculate the concentration values from the logit mP of the samples.

Performance Characteristics

Precision

The intra-assay CVs have been determined at all points on the standard curve. These data are summarized in the graph of Figure 7 (see page 21) and in tabular form on page 20.

Z'-Factor

Z'-factor is a term used to describe the quality of an assay,[4] which is calculated using the following equation:

$$Z' = 1 - \frac{3s_{c+} + 3s_{c-}}{|m_{c+} - m_{c-}|}$$

The theoretical upper limit for the Z′-factor is 1.0. A robust assay has a Z′-factor >0.5. The Z′-factor for Cayman's PGE_2 FPIA - Red was determined to be 0.71.

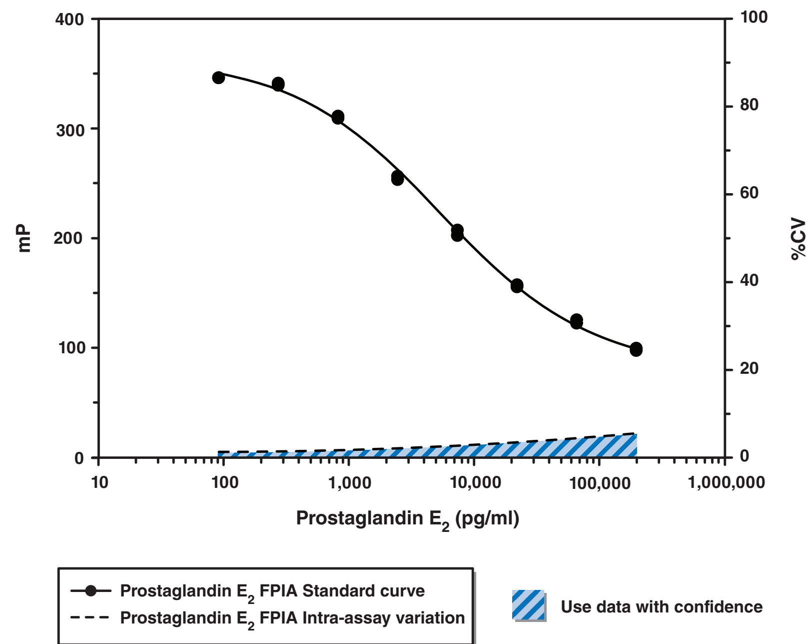
Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

Dose (pg/ml)	mP		Average mP	%CV*
200,000	99	97	98	5.36
66,666	125	122	124	4.63
22,222	155	157	156	4.07
7,407	202	207	205	2.20
2,469	253	256	255	2.31
823	309	311	310	1.55
274	341	339	340	1.31
91	346	346	346	1.48

Table 3. Typical data for the PGE_2 standard curve

*%CV represents the variation in concentration (not mP) of each point of the standard curve.



Average mP_{max} = 350 mP
Detection Limit = 100 pg/ml

Figure 7. PGE_2 standard curve fit to a 4-parameter logistic equation

Specificity

Compound	Cross Reactivity
Prostaglandin E_1	100%
Prostaglandin E_2	100%
Prostaglandin E_2 Ethanolamide	100%
Prostaglandin E_3	85%
Sulprostone	9%
6-keto Prostaglandin $F_{1\alpha}$	2.9%
8-*iso* Prostaglandin $F_{2\alpha}$	0.09%
Prostaglandin D_2	<0.01%
8-*iso* Prostaglandin E_2	<0.01%

Table 2. Specificity of the PGE_2 Monoclonal Antibody

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
High background mP	A. High protein concentration in sample matrix B. Fluorescent molecules in sample matrix	A. Test sample matrix for interference before running samples in the assay

Additional Reading

Go to **www.caymanchem.com/10004517/references** for a list of publications citing the use of Cayman's PGE_2 FPIA Kit - Red.

References

1. Granström, E., Hamberg, M., Hansson, G., *et al.* Chemical instability of 15-keto-13,14-dihydro-PGE_2: The reason for low assay reliability. *Prostaglandins* **19**, 933-945 (1980).
2. Hamberg, M. and Samuelsson, B. On the metabolism of prostaglandins E_1 and E_2 in man. *J. Biol. Chem.* **246**, 6713-6721 (1971).
3. Fitzpatrick, F.A., Aguirre, R., Pike J.E., *et al.* The stability of 13,14-dihydro-15-keto-PGE_2. *Prostaglandins* **19**, 917-931 (1980).
4. Zhang, J.-H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *Journal of Biomolecular Screening* **4(2)**, 67-73 (1999).

Related Products

Prostaglandin D_2 FPIA Kit - Green - Item No. 500581
Prostaglandin D_2 FPIA Kit - Red - Item No. 10007835
Prostaglandin E Metabolite EIA Kit - Item No. 514531
Prostaglandin E_2 - Item No. 14010
Prostaglandin E_2 Affinity Column - Item No. 414018
Prostaglandin E_2 Affinity Purification Kit (4 ml) - Item No. 500450
Prostaglandin E_2 Affinity Purification Kit (20 ml) - Item No. 514018
Prostaglandin E_2 Affinity Sorbent - Item No. 414020
Prostaglandin E_2 EIA Kit - Item No. 514010
Prostaglandin E_2 Express EIA Kit - Item No. 500141
Prostaglandin E_2 FPIA Kit - Green - Item No. 500501
Prostaglandin Screening EIA Kit - Item No. 514012
UltraPure Water - Item No. 400000

Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material **will meet our specifications at the time of delivery**. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

NOTES

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©01/25/2012, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.